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Vol. 31 September, 1954 No. 5

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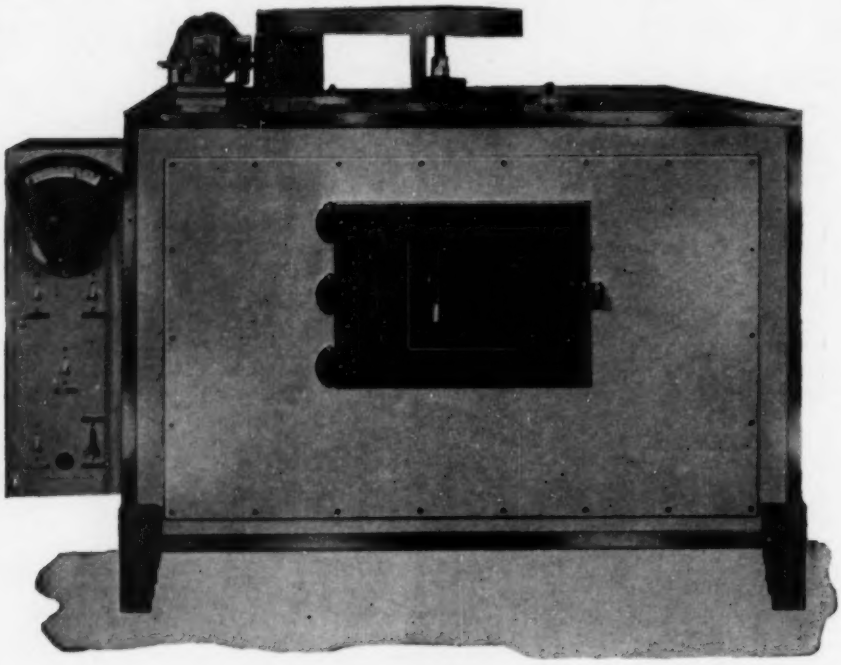

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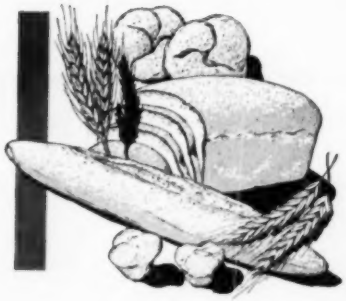


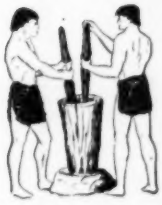
A Brief History of Milling and Baking by **SCIENCE WRITER**

CHAPTER II. *The Beginning of Grain Cultivation and Grinding*

THE GREATEST REVOLUTION

Of all the revolutions through which man has passed, the most profound is that which changed him from a food-gatherer to a food-raiser. In short, man learned to become a farmer and food-processor. There is a clear line down through ages of time showing how man adapted himself to new conditions to support life.

EARLY GRINDING TOOLS



Archeological discoveries in Europe show that man used stones as implements for grinding, rubbing, or pounding. 75,000 years ago these rounded stones with a flat working surface were in use. In the long transition which covered millenniums, man developed mortar forms which were more efficient for grinding because they contained the particles. Actual samples of these devices have been found and dated by experts.

In America, too, pounding stones and mortar-and-pestle forms have been found in once fertile areas, which supported cultures for thousands of years, in California and the Southwestern United States.

COOKING AND BAKING

Caves in France have been discovered where men lived 25,000 years ago. In these crude homes, evidence has been found that milling was done. Hollowed stones bearing the marks of fire and primitive underground ovens in these caves show that these early men cooked foods made from grain, for these devices would not have been necessary to cook only meat.

HARVESTING TOOLS

12,000 years ago, European man had developed small flints with a saw-tooth edge. Probably these were set end-to-end in a stick and the whole device then was used as a sickle for harvesting.

GRAIN CULTIVATION BEGINS

These rubbing stones, these tiny, saw-toothed flints, crude ovens, and fire-stained stone bowls show that man was becoming ready to cultivate the grains. The logical then happened: grain cultivation began. Probably this occurred about 9,000 years ago in a region which is now in Israel and Syria. And it is probable that all ancient wheat cultivation spread from this area.

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*This is the title of a definitive history of milling by John Storck and Walter Dorwin Teague, published by the University of Minnesota Press at Minneapolis and copyrighted by the University of Minnesota. It is used with permission as a source of material for this series of advertisements.

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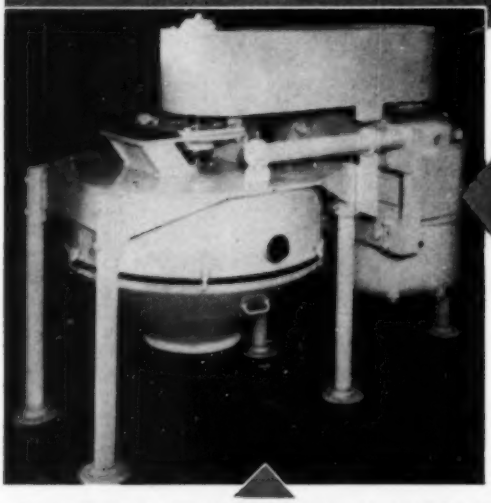

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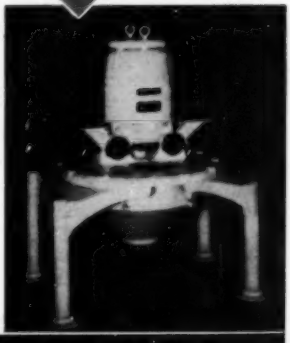

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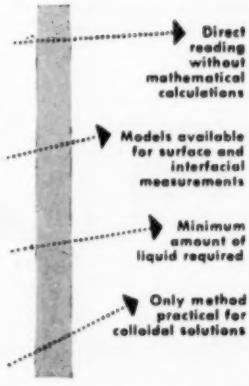
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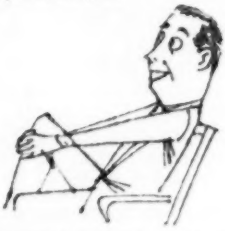

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STUDIES ON THE INCORPORATION OF NONFAT MILK SOLIDS IN WHOLE WHEAT BREAD. I. DOUGH PROPERTIES AND BAKING TESTS[1]

BARBARA M. KENNEDY, LORAINE R. FLETCHER, AND ADELIA R. SABISTON

ABSTRACT

Whole wheat bread containing up to 22% added nonfat milk solids of good baking quality was baked from seven commercial flours and from ten experimentally milled varieties of wheats. The commercial flours tolerated up to 22% milk solids without loss of volume. Optimum volumes were obtained with from 6 to 22% milk solids, depending upon the flour. Volume increases, as compared with water controls, ranged up to 14%.

Of the wheat varieties, Baart, a white spring, and Turkey and Comanche, hard red winters, gave increased volumes with all levels of milk to 22%, while Baart 38, a white wheat, and Wichita, a hard red winter, showed small increases through the 18% levels. Bunyip 41, a white wheat, and Pawnee, a hard red winter, showed volume increases with 6 and 12% milk. Mida and Thatcher, hard red spring wheats, tolerated up to 22% milk solids without loss of volume, while Ramona 44, a white wheat, gave decreased volumes with all levels of milk.

Recent studies on prevailing bakery practices (3, 6, 16) have indicated that commercial white bread contains from three to four parts (range from 0 to 10 parts) nonfat milk solids per 100 parts of flour. Platt (12) reported that a good quality white bread may be made which contains about 25 parts nonfat milk solids or 35 parts whole milk solids per 100 parts of flour. Jack and Haynes (8) reported a study which indicated that the consumption of bread increased as the milk solids in the bread were increased (up to 14%).

More limited studies have been made on the incorporation of milk solids into whole wheat bread. The works of Light and Frey (9), Mitchell *et al.* (10), and Riggs *et al.* (13) indicate that milk also improves the nutritive properties of whole wheat bread, and that whole wheat milk bread produces better growth in experimental animals than does white milk bread. A study by Crespo in 1951 (3) on 23 samples of commercial whole wheat bread showed that an average of 2.4 parts (range from 0 to 5.7) of milk solids was used per 100 parts of flour. No work has been reported on the baking problems arising from the incorporation of milk solids into whole wheat bread.

Because of increased consumer interest in a good quality whole wheat bread of superior nutritive value, the present investigation was undertaken to determine the effect of various levels of a good baking

[1] Manuscript received January 5, 1954. Department of Home Economics, University of California, Berkeley, California.

milk on the dough-handling properties and on the loaf volume of bread made from several varieties of whole wheat flours.

Materials and Methods

Materials. Baking tests were performed on 21 different samples of flour. These included: one commercially blended spring and winter wheat, 80% patent flour; one commercial blend of spring and winter wheat, 95% patent flour; seven commercial whole wheat flours; and 12 samples of known, single-variety, whole wheat flours. A description of the commercial flours is given in Table I. The wheat varieties of differing types, listed in Table II, were experimentally milled into whole wheat flour.

TABLE I

DESCRIPTION OF COMMERCIAL FLOURS

Flour	Grade	Type	State Where Grown	Bromated	Bleaching Treatment
A	80% Patent	Blend of spring and winter	Montana and Washington	No	Bleached
E	95% Patent	Blend of spring and winter	Montana and Washington	Yes (1.7 p.p.m.)	Bleached
F	Whole wheat, Earle process	Hard winter	Kansas and Nebraska	No	Unbleached
G	Whole wheat	Hard spring	Montana	Yes	Unbleached
H	Whole wheat	Hard red spring	Montana	No	
S	Whole wheat	Spring	Montana	No	Chlorine dioxide
X	Whole wheat	Hard red spring	...	No	Chlorine dioxide
K	Whole wheat	Spring	Montana	No	Unbleached
M	Whole wheat	Spring	Montana	No	Bleached

TABLE II

DESCRIPTION OF WHEAT VARIETIES

Variety	Where Grown	Type	Type of Mill in Which Ground
Turkey	Idaho	Hard red winter	Buhr
Baart	Washington	White spring	Buhr
Bunyip 41	California	White	Wolf
Baart 38	California	White	Wolf
Wichita	Kansas	Hard red winter	Buhler
Pawnee	Kansas	Hard red winter	Buhler
Comanche	Kansas	Hard red winter	Buhler
Ramona 44	California	White	Wolf
Mida	North Dakota	Hard red spring	Wolf
Thatcher	North Dakota	Hard red spring	Wolf

The fineness of grind of each flour was determined by sifting 100 g. in a series of 8-in. copper W. S. Tyler standard wire sieves. The sieves, graduated from 42 to 150 meshes to the inch, were shaken on a mechanical Ro-tap sieve shaker for 5 or for 10 minutes, and the amount of flour left on each sieve was weighed.

The water absorption of the flours was determined as g. of water per 100 g. giving a dough that cleared the sides and bottom of the mixer, was neither tight nor slack, and could be handled easily.

The diastatic activity as indicated by the maltose value (1) was determined on about one-half of the flour samples. Although the levels of activity in the single-variety flours were somewhat below those of the commercial flours, they were within an allowable range for fermentation, and no diastase was added.

Protein and ash content, water absorption, maltose value, and particle size of the flours are summarized in Table III.

TABLE III
SUMMARY OF FLOUR CHARACTERISTICS

Flour	Protein[a] N × 5.7	Ash[a]	Absorption[a]	Maltose Value, Maltose/10 g flour[a]	Sieve Test, Shaken 10 Min.			
					Over 42	Through 42, over 80	Through 80, over 100	Through 100
	%	%	%	mg.	%	%	%	%
	Commercial[b]							
A[c,d]	11.3	0.39	62.5	...	0	0	1	93
E[c]	12.1	0.46	64.0	249	0	70	0	27
F[c]	11.9	1.72	68.5	...	13	62	0	20
G[c]	13.7	1.64	69.0	...	11	58	1	29
H[c]	13.8	1.66	68.0	258	5	51	1	40
					4	34	8	61
S	14.5	1.46	64.0	251	12	6	4	75
X	14.8	1.40	58.0	279	8	17	11	60
K[c]	14.8	1.60	68.5	...	12	30	0	55
M[c]	15.1	1.60	68.5	252	9	55	0	34
	Whole wheat varieties							
Turkey sample No. 1[c]	8.4	1.58	56.0	...	48	38	0	13
Baart sample No. 2[c]	10.2	1.77	59.5	...	31	51	1	12
Bunyip 41	10.4	1.65	61.0	182	32	25	6	37
Turkey sample No. 2[c]	10.7	1.48	56.5	...	29	30	2	36
Baart 38	11.3	1.61	59.0	140	18	23	4	53
Wichita	11.3	1.80	62.0	156	12	29	17	44
Baart sample No. 1[c]	11.8	1.60	60.5	...	35	45	1	12
Pawnee	12.1	1.76	65.5	170	6	12	5	75
Comanche	12.3	1.90	65.0	195	7	11	8	72
Ramona 44	12.4	1.48	60.5	209	16	23	7	53
Mida	14.8	1.58	62.5	...	11	18	8	62
Thatcher	15.9	1.52	64.0	...	21	40	6	33

[a] 14% Moisture basis.
[b] A, 80% patent, spring and winter; E, 95% patent, spring and winter; F, whole wheat, hard winter; G, whole wheat, hard spring; H, whole wheat, hard red spring; S, whole wheat, spring; X, whole wheat, hard red spring; K, whole wheat, spring; M, whole wheat, spring.
[c] Sieve test shaken 5 minutes.
[d] 25-g. Sample taken for sieve test.

The sample of spray-dried nonfat dry milk solids used in all the tests was a commercial milk especially prepared for bread baking, which had shown good water absorption and baking qualities in this laboratory.

Experimental Baking Test. For the patent flours, standard test baking procedures (1) were employed with some modifications. The baking formulas and procedures used for the whole wheat flours were adapted from the method of Bohn and Machon (2), and are shown in Table IV. The dry milk was added to the flour. Salt and sugar were

TABLE IV

BAKING INGREDIENTS AND PROCEDURE

Dough Ingredients and Amount Used		Procedure	White Flour	Whole wheat flour	
				Yeast (2%)	Yeast (4%)
	%		*min.*	*min.*	*min.*
Flour[a]	100	Fermentation at 82°F.			
Yeast:		first punch after	165	90	45
white flour	2	second punch after	40	45	30
whole wheat flour	2, 4	sheet and mold	15	15	15
Sucrose	4				
Shortening	2	Total	220	150	90
Salt	2				
Nonfat milk solids	0-24	Proof at 82°F.	75	55	55
Water, variable		Bake at 30°F.	20	20	20

[a] 14% Moisture.

thoroughly blended with the fat, and a weighed portion was added to the milk and flour. A double portion of the dough formula was mixed in a National Complete Mixer which has a four-arm planetary mixing head and two stationary arms. Doughs were mixed until they were of optimum consistency; at 48 r.p.m. this was 3 minutes when no milk solids were added. With the addition of increased amounts of milk solids, the mixing times were increased up to 30 minutes, as shown in Tables V and VI.

In most cases, one part, by weight, of water was added with each part of milk. However, the 95% patent flour and some varieties of whole wheat flours, when combined with 16% or more nonfat milk solids, produced doughs which were too soft and sticky to handle in these proportions. Accordingly the amount of water per part of milk solids was reduced (see Tables V and VI).

The doughs were punched by machine and molded by hand. Duplicate loaves, scaled to 150 g. each after the second fermentation period, were baked from each mix. The series of bakes on a flour with various levels of added milk solids was performed on the same day. In many cases the series was repeated on another day.

TABLE V

EFFECT OF NONFAT MILK SOLIDS ON BREAD MADE FROM COMMERCIAL FLOURS

Flour	Yeast[a]	Nonfat[a] Milk Solids	Water Absorption of Milk	Mixing Time (48 r.p.m.)	Dough Characteristics After Mixing	Volume of Bread	Volume Increase Over Water Control
	%	%	*g H_2O/g milk*	*min.*		*cc.*	%
A	2	0	–	3	satisfactory	552	–
		6	1.00	6	satisfactory	622	13
		12	1.00	9	satisfactory	598	8
E	2	0	–	4	satisfactory	630	–
		6	0.92	5½	satisfactory	655	4
		12	0.83	6¾	very sticky	675	7
F	4	0	–	4	slightly sticky	508	–
		6	1.00	12	satisfactory	535	5
		12	1.00	20	satisfactory	518	2
		16	1.00	24	slightly sticky	555	9
		18	1.00	27	slightly sticky	520	2
		22	1.00	28	slightly sticky[b]	[c]	
G	2	0	–	4	satisfactory, soft	425	–
		6	1.00	8	satisfactory	430	1
		12	1.00	15	slightly sticky	415	–2
	4	0	–	3	satisfactory	–	
		6	1.00	9	satisfactory	432	
		12	1.00	15	satisfactory	455	
		16	1.00	20	satisfactory	448	
		18	1.00	27	sticky[c]	385	
		22	1.00	30	sticky[c]	405	
H	4	0	–	3	satisfactory	518	–
		6	1.00	8	satisfactory	530	2
		12	1.00	13	satisfactory	570	10
		16	1.00	18	satisfactory	578	12
		18	1.00	22	satisfactory	530	2
		22	1.00	28	slightly sticky[d]	538	4
S	2	0	–	3	satisfactory	495	–
		6	1.00	8	satisfactory	518	5
		12	1.00	11	satisfactory	512	3
		16	1.00	14	satisfactory	510	3
		18	1.00	17	satisfactory	505	2
		22	1.00	20	slight sticky	500	1
	2.5	0	–	3	satisfactory	500	–
		6	1.00	8	satisfactory	532	6
		12	1.00	12	satisfactory	515	3
		16	1.00	15	very slightly sticky	518	4
		18	1.00	18	slightly sticky	522	4
		24	1.00	22	sticky[c]	520	4
	4	0	–	3	satisfactory	568	–
		6	1.00	8	satisfactory	600	6
		12	1.00	12	satisfactory	595	5
		16	1.00	15	satisfactory	590	4
		18	1.00	18	satisfactory	568	0
		24	1.00	22	slightly sticky	562	–1
X	2	0	–	3	satisfactory	425	–
		6	1.00	7	satisfactory	468	10
		12	1.00	13½	satisfactory	482	13
		16	1.00	16	satisfactory	485	14
		18	1.00	18¼	slightly sticky	485	14
		22	1.00	22	sticky	485	14
K	2	0	–	4	satisfactory, soft	448	–
		6	1.00	9	satisfactory, soft	472	5
		12	1.00	15	slightly sticky, soft	482	7
M	4	0	–	3	satisfactory	482	–
		6	1.00	8	satisfactory	512	6
		12	1.00	14	satisfactory	508	5
		16	1.00	19	satisfactory	522	8
		18	1.00	23	satisfactory	512	6
		22	1.00	27	slightly sticky	510	6

[a] Flour basis.
[b] Very sticky in sheeter.
[c] Sticky in sheeter.
[d] Slightly sticky in sheeter.
[e] Not baked.

TABLE VI

EFFECT OF NONFAT MILK SOLIDS ON BREAD MADE FROM WHOLE WHEAT FLOURS MILLED FROM INDIVIDUAL VARIETIES

Flour	Yeast[a]	Nonfat[a] Milk Solids	Water Absorption of Milk	Mixing Time (48rpm)	Dough Characteristics after Mixing	Volume of Bread	Volume Increase Over Water Control
	%	%	*g H_2O/g milk*	*min.*		*cc.*	*Inc. %*
Turkey	2	0		3	satisfactory	245	
sample		6	1.00	8	slightly sticky	270	10
No. 1		12	1.00	15	...	295	20
Baart	2	0		3	satisfactory	340	
sample		6	1.00	8	sticky	388	14
No. 2		12	1.00	13	very sticky	400	18
	2	0		3	satisfactory	365	
		6	1.00	8	sticky	393	8
		12	1.00	13	very sticky[e]	415	14
	4[b]	0		3	satisfactory	465	
		6	1.00	8	sticky	522	12
		12	1.00	10	very sticky	538	16
	4	0		3	satisfactory	355	
		6	1.00	9	satisfactory	418	18
		12	1.00	16	sticky	428	21
		16	1.00	30	very sticky		
		16	0.75	15	sticky	450	27
		18	0.78	25	very sticky	352	— 1
		22	0.82	20	sticky	405	14
Bunyip 41	2	0		3	satisfactory, soft	378	
		6	1.00	8	satisfactory, soft	408	8
		12	1.00	14	sticky[f]	385	2
		16	1.00	16	very sticky[f]	368	— 3
		18	0.89	19	very sticky[f]	320	—15
		22	0.86	20	very sticky[g]	330	—13
Turkey	2	0		3	satisfactory	318	
sample		6	1.00	6	slightly sticky	335	5
No. 2		12	1.00	12	sticky	350	10
	4	0		3	satisfactory	320	
		6	1.00	7	satisfactory	372	16
		12	1.00	18	sticky[f]	382	19
		16	1.00	20	sticky[f]	382	19
		18	1.00	23	sticky[f]	370	16
		22	1.00	25	sticky[f]	350	10
	4	0		3	satisfactory	338	
		6	1.00	6	satisfactory	375	11
		12	1.00	13	slightly sticky	382	13
		16	1.00	17	slightly sticky	388	15
		18	1.00	20	slightly sticky	378	12
		22	1.00	25	sticky	345	2
Baart 38	2	0		3	satisfactory	372	
		6	1.00	6	satisfactory	420	13
		12	1.00	11	sticky[f]	428	15
		16	1.00	13	sticky[f]	390	6
		18	0.83	15	sticky[f]	382	3
		22	0.86	17	sticky[g]	360	— 3
Wichita	2	0		3	satisfactory	438	
		6	1.00	8½	satisfactory	460	5
		12	1.00	14	very slightly sticky	468	7
		16	1.00	18½	slightly sticky	445	2
		18	1.00	21	slightly sticky	458	5
		22	1.00	23	sticky	430	— 2
Baart	2	0		3	satisfactory	382	
sample		12	1.00	15	sticky	420	10
No. 1		6[c]	1.00	6	satisfactory	422	10
		12[c]	1.00	12	satisfactory	458	20
	4	0		3	satisfactory	425[d]	
		6	1.00	8	satisfactory	468[d]	
		12	1.00	16	slightly sticky	445[d]	
		16	1.00	17	sticky	450	
		18	1.00	20	sticky	475	
		22	1.00	20	sticky	488	

TABLE VI—CONTINUED

EFFECT OF NONFAT MILK SOLIDS ON BREAD MADE FROM WHOLE WHEAT FLOURS MILLED FROM INDIVIDUAL VARIETIES

Flour	Yeast[a]	Nonfat[a] Milk Solids	Water Absorption of Milk	Mixing Time (48rpm)	Dough Characteristics after Mixing	Volume of Bread	Volume Increase Over Water Control
	%	%	*g H_2O/g milk*	*min.*		*cc.*	*Inc. %*
Pawnee	2	0		3	satisfactory	448	
		6	1.00	8		472	5
		12	1.00	11½	slightly sticky	462	3
		16	1.00	15	sticky, soft	442	− 1
		18	0.92	17	very sticky, soft	422	− 6
		22	0.91	19	very sticky, soft	420	− 6
Comanche	2	0		3	satisfactory	440	
		6	1.00	8	satisfactory	460	5
		12	1.00	14	satisfactory	470	7
		16	1.00	18½	satisfactory	478	9
		18	1.00	20	satisfactory	448	2
		22	1.00	22½	sticky	465	6
Ramona 44	2	0		3	satisfactory	438	
		6	1.00	6	slightly sticky	432	− 1
		12	1.00	14	sticky[f]	380	−13
		16	0.81	10	sticky[f]	388	−11
		18	0.83	14½	sticky[f]	353	−19
		22	0.73	17	sticky[f]	335	−24
Mida	2	0		3	satisfactory	492	
		6	1.00	6	satisfactory	492	0
		12	1.00	9	satisfactory	505	3
		16	1.00	12	satisfactory	498	1
		18	1.00	14	satisfactory	488	− 1
		22	1.00	18	sticky	480	− 2
Thatcher	2	0		3	satisfactory	485	
		6	1.00	6	satisfactory	490	1
		12	1.00	9	slightly sticky	485	0
		16	1.00	16	sticky[h]	488	1
		18	1.00	16	slightly-sticky	488	1
		22	1.00	20	sticky	490	1

[a] Flour basis.
[b] Fermentation time 150 min.
[c] Different brand of milk.
[d] Fermentation cabinet temperature low, proof time increased.
[e] One lost in sheeting.
[f] Easily handled in sheeter.
[g] Still sticky in sheeter.
[h] Slightly sticky in sheeter.
[i] Not baked.

The bread was baked in standard low-form tins. In preliminary experiments, the loaves were baked for 25 minutes at 430°F. but the whole wheat loaves, with their somewhat lower volume as compared with white breads, appeared overbaked, with thick, dark crusts. A reduction in baking time to 20 minutes gave satisfactory loaves. The loaves were weighed, and the volume was measured 1 hour after they were removed from the oven.

The loaf volumes of the majority of the duplicate loaves agreed within 15 cc. or less. When an occasional duplicate varied by more than 25 cc. the bake was repeated. All loaf volumes reported in this paper, unless otherwise indicated, are the average of two loaves from the same mix.

Loaves were examined on the following day for grain and texture. Changes in these properties appeared to correlate closely with changes

in loaf volume. No attempt was made to score the loaves numerically.

Results and Discussion

The effects of increasing amounts of nonfat milk solids on the water absorptions, mixing times, and handling properties of the doughs, and on the loaf volumes of bread made with commercial white and whole wheat flours, are shown in Table V. Similar data for bread made with whole wheat flours milled from individual varieties are given in Table VI. These data show that the whole wheat breads can tolerate considerable quantities of milk and also that the loaf volumes were increased with most of the flours used.

Water Absorption. With all of the commercial flours except flour E, one part of water, by weight, was added for each part of milk when up to 22% milk solids were added. With flour E, a 95% patent, it was necessary to reduce the amount of water, and at the 12% milk level the reduction should have been greater to prevent stickiness of the mixed dough. It will be noted from the table that, with a 1:1 proportion of water to milk, the doughs containing 22% milk, and occasionally the 18% milk doughs, were sticky after mixing. In some cases, where the stickiness was not pronounced, the doughs became drier after fermentation and could be easily handled in the sheeter. Where the doughs were still sticky after fermentation, they could be sheeted if the rollers were lightly greased.

With all of the varietal flours used, one part of water per part of milk was added through 12% milk. With Baart sample No. 2 and Ramona 44, the water was reduced starting with the 16% milk doughs, and with Bunyip 41, Baart 38, and Pawnee, at 18% milk. It was possible to handle the 1:1 proportion of water to milk in doughs containing 22% milk solids with the varieties Turkey, Wichita, Baart sample No. 1, Comanche, Mida, and Thatcher. Although many of the doughs were sticky after mixing, particularly when 12% or more milk was used, the doughs firmed up after fermentation, were dry to the touch, soft and pliant, and were easily handled in the sheeter. This was particularly noted in the cases of Turkey sample No. 2 and Ramona 44, and with all but the 22% milk doughs with Bunyip 41 and Baart 38. With the latter, and in a few other cases, the 22% milk doughs remained sticky after fermentation, and greased rollers were used in sheeting.

Mixing Time and Speed. Doughs were mixed at a speed of 48 r.p.m. until they were of optimum consistency and did not adhere to the mixing bowl. Overmixing, as well as too large amounts of water for

the amount of milk, produced sticky doughs which in some cases were so difficult to handle and sheet that the bakes were not completed. Preliminary experiments with mixing whole wheat flour doughs at 100 r.p.m. gave unsatisfactory results. Even if the doughs were of good handling quality as they came from the mixer, they became dry during fermentation, and the baked loaves were dry and of poor volume. When more water was added to the dough ingredients, the point of optimum gluten development was difficult to determine, and the dough was sticky on coming from the mixer and consequently difficult to handle. For this reason, a pulley arrangement was installed on the mixer, making possible speeds of 48 and 80 r.p.m. With the whole wheat flours and milk, a longer mixing time at 48 r.p.m. produced doughs of much better handling qualities than did a shorter mixing period of 100 r.p.m. The more satisfactory doughs obtained at 48 r.p.m. with the whole wheat flours may have resulted from the longer time required for the flour bran and the milk to become completely hydrated. The firming of the doughs after fermentation would substantiate this. A somewhat lower speed for use with milk was also indicated as a result of work on the effect of the relation between mixing speed and dry milk solids on bread volume by Stamberg and Bailey (14) and by Swanson and Bayfield (15).

As the amount of added milk solids was increased, it became necessary to increase the mixing times. The mixing time, in minutes at 48 r.p.m., was approximately equal to the percentage of nonfat milk solids added. The increase in mixing time when 6% and 12% milk solids are added has been previously observed. Stamberg and Bailey (14) studied the effects of mixing times at various speeds on a strong flour and on a medium-strength flour, without milk and with 6% high-quality dry milk solids. They concluded that moderately high mixing speeds (60 and 80 r.p.m.) resulted in superior bread, as judged by baking scores, and that with reduced speeds (16 and 40 r.p.m.), the bread was inferior even when optimum time of mixing for a given speed was allowed. They pointed out that weaker flours may easily be overmixed at the higher speeds (120–160 r.p.m.). These workers mixed 300 g. of flour doughs when 6% milk solids were used, and 319 g. of flour doughs without milk, thus approximately equalizing the weight of dough in the mixer. They reported, as optimum, mixing times of 27 minutes with no milk solids and 28 minutes with 6% milk solids for the strong flour (14.2% protein) at 40 r.p.m.; 23 minutes with no milk and 25 minutes with 6% milk solids for the medium-strength flour (11.1% protein) at the same speed.

Swanson and Bayfield (15) studied three flours with four levels of milk solids (0, 2, 6, and 12%) and with several mixing speeds. Three types of mixers were used. These workers concluded that at all speeds of all machines, optimum mixing time increased with increasing milk solids, but the increase was very small at high speeds. They observed that when twin-loaf doughs were mixed at 50 r.p.m. in the Swanson-Working Mixer, the commercial flour required mixing times of about 3.5, 4, 5, and 8 minutes for the 0, 2, 6, and 12% milk doughs, respectively. Loaf-volume data in this study did not support the conclusion of Stamberg and Bailey that the addition of milk solids increases the tolerance to mixing speed. In fact, it would appear that mixing speed, when the mixing time was controlled, had relatively little effect on the relation between a given level of milk solids and loaf volume with Chiefkan and with a commercial flour. The response of loaf volumes to added dry milk solids varied with the kind of flour, the mixing time and speed, the type of mixer, and the amount of milk solids added.

In the present study 200 g. of flour doughs were used, and the dough weights were not equalized. The mixing times of flours A and B were in accord with those found by Swanson and Bayfield.

Loaf Volume. Table V shows that the breads made from both white and whole wheat commercial flours had increased volumes over those of the water controls when up to 22% of good quality, nonfat milk solids was used in the dough formulas. These volume increases ranged up to 14%. The largest loaf volumes were not found at any one milk level; depending upon the flour, maximum volumes were obtained with from 6 to 22% added milk solids.

Three of the whole wheat flours, F, H, and M, gave the largest volumes with 16% milk solids with the 4% yeast formula—increases of 9, 12, and 8%, respectively. Flour S had the greatest volume—increases of about 5%—with 6% milk solids using the 2, 2.5, and 4% yeast formulas. With the 2% yeast formula, flour G had the largest volume with 6% milk solids, 1% increase; flour K, with 12% milk solids, 7% increase; and flour X, with 16 through 22% milk solids, 14% increase, with the amounts of milk tried. In all cases, with both commercial and experimental flours, the textures of the milk breads were as good as, or better than, those of the water controls.

The reliability of the differences in loaf volume when commercial whole wheat flour H was baked with different quantities of the good baking milk is shown in Table VII. From 4 to 6 bakes, at 4 milk levels, were performed on this flour, each series being baked on a dif-

TABLE VII

ANALYSIS OF VARIATIONS IN LOAF VOLUME OF BREAD BAKED FROM COMMERCIAL WHOLE WHEAT FLOUR H WITH ADDED NONFAT MILK SOLIDS[a]

Nonfat Milk Solids, Flour Basis	Bakes	Mixing Time (48 r.p.m.)	Loaf Volume Av.	Paired Differences: Increase over 0% Milk		*t*—Value	*P*
%	*No.*	*min.*	*cc.*	*cc.*	%		
0	6	3	518	...	...	...	...
6	6	7–8½	536	18	3.5	6.35	0.01
12	6	11–14	560	41	8.1	8.28	0.01
16	4	18–19	571	62	14.9	7.11	0.01
18	5	22–23	552	42	8.5	2.97	0.02
22	4	28–30	546	38	7.5	4.68	0.01
12–6	6	...	...	...	...	3.19	0.02
16–12	4	...	...	...	...	3.24	0.05
18–16	4	...	...	...	...	1.83	0.2
22–18	4	...	...	...	...	0.29	0.8

[a] Formula contained 4% yeast; 1 part water to 1 part milk throughout.

ferent day. Because of slight daily variations in baking conditions, *t* values were calculated on the paired differences. The volumes of all the milk breads were significantly higher than those of the water controls. The volumes of the loaves with 6 and 12% milk, and those with 12 and 16% milk, showed differences at the 2% and 5% levels of significance, respectively.

With the varietal flours (Table VI) there was considerably more variation in volume at the different milk levels than with the commercial flours. Baart, Turkey, and Comanche gave increased volumes with all levels of milk through 22%, while with Baart 38 and Wichita, small increases were noted through the 18% milk levels. Bunyip 41 and Pawnee showed volume increases with 6 and 12% milk. Mida and Thatcher tolerated 22% milk solids without loss of volume, while Ramona 44 gave decreased volumes with all levels of milk.

There was a tendency for the white wheat flours to have a lower protein content and a coarser grind, to give lower loaf volumes with the water controls, and to show greater improvement in loaf volume when milk solids were added than in the case of the red wheats. An exception to this trend in the white wheats was Ramona 44. This is a California wheat not usually used for yeast breads. A relatively fine-grind flour of 12.4% protein, which gave a loaf volume of 438 cc. for the water control, it gave volume decreases at all the milk levels, the volume becoming lower as the amount of milk solids was increased. It was necessary to decrease the proportion of water to milk with this flour, and although the doughs were sticky after mixing, they were

easily handled in the sheeter after fermentation.[2]

The red wheats, with the exception of the two samples of Turkey, all had loaf volumes with the water control of 430 cc. or over, using the 2% yeast formula, and gave moderate to no increase in volume when milk was added. Pawnee was the only red wheat with which it was necessary to reduce the proportion of water to milk, and this with the 18 and 22% milk doughs only. Volume decreases of 6% were associated with these doughs. Mida and Thatcher showed relatively little volume change at any of the milk levels. The proportion of one part of water to one part of milk was maintained throughout. Although no significant increases in volume were noted with the added milk, 22% milk solids could be carried in both flours with no loss in volume.

The two samples of Turkey, a hard red winter wheat grown in Idaho, were low in protein (8.4 and 10.7%) and had a fairly coarse grind (Table III). Both samples had improved loaf volumes with 6 and 12% nonfat milk solids. Sample No. 2 was studied more extensively. A 1:1 proportion of water to milk could be used throughout with good dough handling properties after fermentation. Increased loaf volumes were obtained through 22% milk, the largest volume being obtained with 16% milk—increases of 15 and 19%.

A review of the literature with reference to the effect of nonfat dry milk solids on the loaf volume of bread made from white flour indicates that when no shortening is in the baking formula, milk solids decrease the loaf volume, and that when shortening is used a good baking milk increases the volume. That the presence or absence of shortening explains the difference in loaf volume when nonfat milk solids are added to a formula has been reported by Eisenberg (4). He compared loaves baked with no milk and with 6% nonfat milk solids, with no shortening and with 3% shortening in the formula, at various levels of bromate. His data showed that milk without shortening depressed the loaf volumes, but that milk with shortening increased them. He emphasized the importance of shortening in milk formulas. In the present study on whole wheat flours, 2% shortening was used throughout.

Effect of Bromate. It is recognized that the amount of bromate required to produce optimum texture and the greatest loaf volume varies with the variety of wheat and with the addition of milk. However, in view of the large number of flours used in the present study,

[2] This reduction in water with 16% or more milk solids was necessary with most of the white wheats, Baart sample No. 1, Bunyip 41, and Baart 38. It might be noted that most of the cases of greatest volume depression occurred when the proportion of water to milk solids was decreased.

each baked into loaves at several different levels of added milk solids, it was considered impractical to determine the optimum bromate level in each case. For this reason, except for flour S, no bromate was used in the bread formulas, and the comparisons between the different milk levels were made on a no-bromate basis.

That such comparisons are valid is supported by the work of Ofelt and Larmour (11). They noted that, with the addition of 6% milk solids to commercial white flours, the optimum amount of bromate was found to be, in most cases, greater than that required for the water controls. It was noted that direct comparisons of milk-free and milk doughs at any one level of bromate dosage are bound to be misleading, but that perhaps the most admissible comparisons would be on the nonbromated doughs. These workers also noted that, in certain flours, greater improvements were obtained with milk alone than with the optimum dosage of bromate without milk.

Because of the relationship between bromate and milk reported by various workers (11, 4, 7, 17) which indicated that potassium bromate improved the loaf volume of some flours when 6% nonfat milk solids were added, one of the unbromated, commercial whole wheat flours, flour S, was baked with 0, 6, 12, and 18% added nonfat milk solids, with varying amounts of potassium bromate at each milk level. Both the 2% and the 4% yeast formulas were used. A series with the different amounts of milk and no bromate was baked on one day while the bromate series with a given level of milk were run on different days. Ten baking days were required for the entire series, results of which are given in Table VIII. There is no indication that the bromate requirement was increased with the increase in milk solids. The bromate gave greater volume increases at a given milk level with the 2% yeast formula than it did with the 4% yeast formula. The data with this flour indicated that the percentage of milk solids and the amount of yeast produced changes in loaf volume of greater magnitude than those effected by the bromate. These data further substantiate the observations of Ofelt and Larmour (11) and Finney and Barmore (5) that milk solids serve to buffer the detrimental effects resulting from overdosages of bromate.

Baking Time and Temperature. Platt (12) baked white bread with 35% added whole milk solids at 375°F. Although all the loaves in the present study were baked at 430°F. it would have been more desirable, particularly as regards crust color, to have baked the higher percentage milk breads at a lower temperature. At 430°F. the crusts of the breads containing 18% and 22% milk were too dark. For good oven

TABLE VIII

EFFECT OF NONFAT MILK SOLIDS AND BROMATE ON THE VOLUME OF BREAD MADE FROM COMMERCIAL WHOLE WHEAT FLOUR S

	Mg. of $KBrO_3$/100 g Flour[b]							
Milk Solids	0[a]	0	1	2	3	4	5	6
%	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
	4% Yeast Formula							
0	518	513	510	520	520	528	510	...
6	568	570	...	553	563	565	568	555
12	595	563	575	578	568	565	565	...
18	568	583	588	555	560	563	553	...
	2% Yeast Formula							
0	448	445	450	450	458	450	430	...
6	480	450	468	450	493	498	480	...
12	493	488	503	503	508	515	513	...
18	488	478	480	473	490	510	495	...

[a] Each yeast formula with no bromate but different milk levels baked on a separate day.
[b] Each % milk with varying amounts of $KBrO_3$ baked on a separate day.

spring and crust color it would be preferable to start at 430°F. and then lower the temperature. However, this was not practical with our experimental procedure. In subsequent work with 12% added milk solids, loaves have been baked from 150 g. of dough at 410°F. for 25 minutes, or at 430°F. for 22 minutes, with good results.

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THE INFLUENCE OF MOISTURE CONTENT AND OTHER FACTORS ON THE STABILITY OF WHEAT FLOURS AT 37.8°C.[1]

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ABSTRACT

The stability of various grades of white and whole wheat flour from a hard wheat mix stored in sealed containers at 3, 6, 10, and 14% moisture and 37.8°C. decreased with decreasing refinement, with treatment with chlorine dioxide, and with an increase in the moisture content.

During 38 weeks storage at 3 and 6% moisture the baking quality of unbleached short patent flour showed little change; at 10 and 14% moisture the loaf volume decreased sharply after 38 and 10 weeks storage respectively. In contrast, whole wheat flour stored at 10 and 14% moisture lost in loaf volume after 26 and 3 weeks storage respectively. Heat treatment of the feed streams entering into whole wheat flour, treatment with 0.01% of two antioxidants, or storage under nitrogen did not retard the losses in breadmaking value upon storage at 14% moisture.

Development of objectionable odors was enhanced when the storage moisture was increased from 6 to 10 to 14%. Several samples stored at 3% moisture had more objectionable odors than those at 6%. Bleaching shortened the storage time at which undesirable odors were detected. Feed products acquired rancid odors more rapidly than flours.

Development of free fatty acids increased with decreasing refinement and was not influenced by bleaching. At 3% moisture, there was essentially no increase in fat acidity of the various flours whereas at 14% moisture high percentages of the total lipids were hydrolyzed. Significant negative correlations were obtained between loaf volume and fat acidity but exchanging the lipids of sound and deteriorated flour indicated that other biochemical changes also contributed to the losses in baking quality. Peroxide and monocarbonyl values for the flour lipids were low and showed little change during the storage trials.

Sulfhydryl contents of the flours increased with decreasing refinement but were not significantly influenced by treatment with chlorine dioxide. The sulfhydryl values decreased in the flours stored at 6, 10, and 14% moisture.

Thiamine losses occurred in whole wheat flour after 3 weeks storage at 14% moisture. The red dog and wheat germ streams lost about 80% of their thiamine when stored for 52 weeks at 14% moisture.

The stability of bread flours may be enhanced by increasing the degree of refinement, eliminating treatment with oxidizing improvers, and decreasing the moisture content below normal commercial levels.

The changes which occur in the breadmaking value of wheat flours during storage depend upon several factors such as flour grade,

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extent of treatment with oxidizing improvers, moisture content, and temperature. It is common knowledge among baking technologists that untreated freshly milled flour from a new-crop wheat normally improves in baking quality for a period of time at a rate which increases as the moisture content and temperatures are raised. Upon longer storage a decline in baking quality occurs. This improvement appears to involve oxidative changes in certain flour constituents, since it can be brought about by appropriate treatment with several oxidizing agents. Upon further storage, flour which is artificially matured in this way tends to deteriorate in baking quality more rapidly than untreated flour of the same grade.

While the mechanism of the aging process is not well understood, it has been established that hydrolytic and oxidative changes in the lipids have an important bearing on the keeping quality of wheat flours (4, 15, 19, 20, 22). Kozmin (15), Sullivan, Near, and Foley (22), and Barton-Wright (4) observed that unsaturated fatty acids produced short brittle glutens. However, Sullivan *et al.* (20, 22) demonstrated that a pronounced damaging action on breadmaking properties did not occur unless these acids became oxidized either by air or by the oxidizing enzymes present in the flour.

The development of fat acidity is accelerated by moisture, whereas oxidative rancidity appears to be enhanced when the moisture content of flour is reduced to low levels. Thus, Fine and Olsen (9) found that patent flour containing 5.6% moisture did not become rancid during 3 years' storage at room temperature; but when the moisture content was reduced to 2.0% or lower, the flour became rancid in 2 to 4 months. Halton and Fisher (10) observed that the rate at which flour absorbed oxygen at 60°C. increased as the moisture content was decreased. More recently, Andrews (2) found that bakers' patent flours containing 1.5, 4.0, and 8.0% moisture were rancid after 6 months' storage at 40°C.; organoleptic tests indicated that the extent of rancidity was in inverse relation to the moisture content. Samples of the flour stored at 11.0 and 13.5% were entirely free from rancidity. While the mechanism of flour improvement is unknown, the investigations of Sullivan and her associates (21) and of Baker, Parker, and Mize (3) have pointed to the significance of oxidation and reduction of the sulfhydryl linkage of the flour proteins in relation to the action of oxidizing improvers in breadmaking. More recently Hlynka (12) and Hlynka and Bass (13) have reported that a reaction occurs between the protein and carbohydrate components of flour, which they suggest may be associated with flour maturation.

Several flour storage studies have been reported in the literature but in most cases no attempt was made to follow the biochemical changes during storage. In several instances, moisture content and temperature were not controlled and the results are frequently in conflict. McCalla *et al.* (16) found that the breadmaking value of long patent hard wheat flour stored in sealed containers at 13.5% moisture and 16° to 28°C. fell off quite markedly within 3 months, but when stored in bags the flour improved for 6 months, after which the breadmaking value decreased. Thomas (23) found that flours containing 15.8, 14.5, and 13% moisture held in canisters retained satisfactory breadmaking qualities for 6, 18, and 24 months, respectively. He cited an experiment by Neumann reported in 1921 in which a flour, vacuum-dried to 4.9% moisture, had normal baking quality, color, and odor after 4 years' storage.

In military operations flours frequently must be stored for longer times and at higher average temperatures than in normal civilian use. For this reason, it is desirable that all ration items purchased for military use should yield acceptable food products after storage for at least 6 months (and preferably 12 months) at 100°F. (37.8°C.).

The present study was undertaken to explore the influence of moisture content and artificial maturation on the stability at 37.8°C. of various grades of white and whole wheat flour milled from a hard wheat mix. With whole wheat flour, the effects on stability of treatment with antioxidants, of heat-treatment of the feed streams entering into its composition, and of storage at low oxygen tensions also were investigated. In addition, storage trials were carried out with the various feed streams. After storage in sealed containers at moisture levels of 3, 6, 10, and 14%, the different flours were subjected to various tests including experimental baking, physical properties of the doughs, hydrolytic and oxidative changes in the lipids, and sulfhydryl content. The development of fat acidity was followed in the feed streams and mold counts and thiamine determinations were made on some of the samples.

Materials and Methods

Wheat Products Employed and Their Preparation for Storage. The wheat products obtained for these storage trials are listed in Table I; they were commercially milled from a wheat mix (protein content 13.8% on a 14.0% moisture basis) composed of 85% hard red spring and 15% of Montana hard red winter wheats. The various feed products were pulverized in a hammer mill to pass through a

72-mesh sieve. When received, the different wheat fractions contained about 13% moisture. Subsamples were adjusted to moisture levels of 3, 6, and 10% by drying in a large vacuum oven at 37.8°C. (100°F.); to bring another subsample of each to 14% moisture, the samples were sprayed with water from an atomizer while being agitated in an enclosed mixer. Appropriate fractions at each of the above moisture levels were composited in the proper percentages to give

TABLE I

COMPOSITION OF FLOURS AND FEED PRODUCTS USED IN STORAGE TRIALS[a]

Wheat Product	Bleaching and Maturing Treatment[b]	Mill Yield	Protein	Ash	Lipids[c]
Flours		%	%	%	%
Patent	None	65	12.2	0.42	1.3
	Benzoyl peroxide, chlorine dioxide	65	12.2	0.40	...
First clear	None	6	16.5	0.74	2.0
	Chlorine dioxide	6	...	0.72	...
Second clear	None	3	19.0	1.34	3.3
	Chlorine dioxide	3	18.7	1.34	...
Whole wheat	None	100	13.8	1.66	2.4
	Chlorine dioxide	100	13.5	1.54	...
Feed by-products					
Red dog		1.5	17.4	3.72	4.3
Brown middlings		12.5	16.6	4.54	5.4
Bran		11.5	14.7	6.04	5.5
Germ		0.5	28.5	4.03	12.9

[a] All analytical values are expressed on a 14% moisture basis.
[b] The bleached patent flour was treated with 0.15 g. chlorine dioxide per 100 lb. and also with a commercial bleaching agent containing benzoyl peroxide. Barley malt flour was added to bring the amylase activity to desirable limits. The bleached first and second clear flours were treated with 1.5 g. chlorine dioxide per 100 lb.
[c] The lipids were determined by extraction with diethyl ether followed by absolute ethanol.

long patent (patent + first clear), straight grade (patent + first and second clear), and whole wheat flour (all fractions). The various flours and streams were held at −18°C. until all the samples were ready for storage.

Subsamples of the whole wheat flour were treated with 0.01% by weight of two antioxidants, namely AMIF 72 (a commercial mixture developed by the American Meat Institute Foundation, which contains 20% butylated hydroxyanisole, 6% propylgallate, 4% citric acid, and 70% propylene glycol) and monoisopropyl citrate, added in the form of a 30% solution in propylene glycol. These antioxidants were sprayed on the flour from an atomizer during continuous agitation in a closed container.

Whole wheat flour at 14% moisture was also sealed at low oxygen tension in 1-gal. Mason jars. A small hole was made in the lid of the

jar, which was then successively placed under vacuum and flushed with nitrogen in a large container. This operation was repeated three times and after the last treatment with nitrogen the hole in the lid was rapidly soldered.

To determine the effect of heat treatment of the feed streams which enter into the composition of whole wheat flour on its stability, a blend of the appropriate proportions of pulverized bran, brown middlings, red dog, and germ was mixed with an equal amount of water. The mixture was autoclaved at 110°C. for 1 hour and dried under vacuum to approximately 10% moisture content. This "concentrate" was then blended with appropriate quantities of straight grade flour and the resulting whole wheat flour brought to 14% moisture.

The various samples were all packed and tightly sealed in 1-gal. glass Mason jars.

Storage Trials. About 3 months after the wheat was milled, the sealed Mason jars containing the flours and mill streams adjusted to the different moisture levels were transferred to a constant-temperature room maintained at 37.8°C. Samples of each product at the moisture contents at which they were received from the mill were also stored at —18°C. to serve as controls for the experimental baking tests. After selected intervals, samples were removed from storage for baking tests and various analytical determinations.

Experimental Baking Tests. The different flours were baked by employing 100 g. of flour (14.0% moisture basis). The formula for the whole wheat flour contained 5% sucrose, 1% salt, 3% yeast, 3% shortening, and an appropriate quantity of water. The same formula was used for baking the other flours except that the shortening was omitted. The doughs were fermented 2 hours at 30°C., proofed 1 hour at 30°C., and baked for 25 minutes at 230°C. The baking tests were made with four levels of potassium bromate; namely, 0, 1, 2, and 3 mg/100 g flour. The loaf volume was determined 1 hour after baking and the loaves were scored for external and internal appearance in the usual manner.

Physical Dough Tests. Extensograph tests and measurements of gas production and retention were made on doughs prepared from several of the flour samples after varying periods of storage.

The doughs for the extensograph test contained 2.0% salt (flour basis) and were prepared in the farinograph, the amount of water being adjusted to provide a dough with a consistency of 500 B.U. at maximum development. After mixing, the dough was divided into

three equal parts of 150 g. each. The first dough was rounded, molded, and placed in the saddle immediately after mixing; the other two pieces of dough were kept at 30°C. for 90 minutes after mixing and then were rounded, molded, and placed in the saddle. The three doughs were kept 1 hour in the saddle at 30°C. before they were stretched.

For the determination of gas production and retention, 125 g. of flour, 1.25 g. salt, 3.75 g. yeast, and an appropriate amount of water were mixed in a Swanson mixer (McDuffee bowl) and placed in the dough chamber of the Chopin Zymotachygraphe (5) maintained at 30°C. An automatic record of the gas production and retention values was obtained over a 6-hour period.

Organoleptic Tests. Samples of the various products stored in 16-oz. Mason jars at 37.8°C. were smelled at room temperature at selected intervals by four analysts working independently. Each sample was assigned a numerical grade of 1 to 6, the highest score being given to the samples with the most objectionable odor. After scoring, the samples were stored at —18°C. and retested along with samples subsequently removed from storage at 37.8°C.

Analytical Methods. Moisture was determined by drying a 2.0-g. sample in an air oven at 130°C. for 1 hour as described in Cereal Laboratory Methods (1). Protein content was determined by the modification of the Kjeldahl procedure described in the same manual.

The lipids in the various products were extracted in a Soxhlet with diethyl ether for 3 hours, followed by extraction with absolute ethanol for 4 hours. The solutions were evaporated to dryness under a stream of nitrogen, after which the residues were taken up in diethyl ether; the solutions were then combined, dried with anhydrous sodium sulfate, filtered, and evaporated to dryness under nitrogen on a water bath maintained at 50°C.

The lipids obtained in this manner were analyzed for free fatty acid content, iodine value, peroxide value, and monocarbonyl compounds. Free fatty acids were determined by titration of a solution of the oil in neutral 95% ethanol with 0.1 *N* potassium hydroxide solution using phenolphthalein as indicator. The results were expressed both as fat acidity (mg. potassium hydroxide required to neutralize the free fatty acids in the lipids present in 100 g. of the wheat product) and as free fatty acids (percentage of free fatty acids, expressed as oleic acid, present in the lipid).

Iodine values were obtained by the Wijs method, as described in Cereal Laboratory Methods (1). Peroxide values were first deter-

mined by the iodometric method of Wheeler (24), in which the iodine resulting from the oxidation of potassium iodide by the peroxides in the lipid is titrated with standard thiosulfate solution. After it was found that this method failed to reveal any appreciable differences in the peroxide value of the lipids, a modification of the ferric thiocyanate procedure of Hills and Thiel (11), devised for the estimation of small amounts of peroxide in milk powder, was employed. Two to four grams of the wheat product were refluxed 1 hour with a benzene-methanol mixture (70 + 30 by vol.). After filtration, suitable aliquots of the extract were made up to 10.0 ml. with the benzene-methanol solvent; one drop of ammonium thiocyanate solution (30 g. per 100 ml.), one drop of ferrous chloride solution (0.4 g. hydrated barium chloride, 0.5 g. hydrated ferrous sulfate, and 2.0 ml. conc. hydrochloric acid solution in 100 ml. water), and 0.01 ml. 4 *N* hydrochloric acid solution were added; and the absorbancy was read at 510 mμ.

Since volatile monocarbonyl compounds are among the secondary reaction products which arise from the cleavage of the hydroperoxides that are initially formed in the oxidative deterioration of a fat, Pool and Klose (18) have suggested that they may contribute more to the off-odors in rancid fat than the relatively nonvolatile peroxides. Their method for the estimation of monocarbonyl compounds is based upon the formation of 2,4-dinitrophenyl-hydrazones, removal of any unreacted reagent and of the hydrazones of dicarbonyl compounds on an alumina column, and measurement of the absorbancy of the filtrate at 435 mμ. In the present studies 5.0 g. of flour were extracted with 10 ml. of benzene for 2.5 hours at room temperature, the suspension filtered, and a 2- to 3-ml. aliquot analyzed by the method of Pool and Klose (18). The sulfhydryl content of the samples was determined by the method of Holme and Spencer (14), which is based upon the iodometric oxidation of the sulfhydryl groups in the presence of orthoiodosobenzoate and potassium iodide.

Thiamine determinations were made by the thiochrome procedure (1), on samples of whole wheat flour, red dog, and germ stored for various intervals of time at the four moisture levels.

Mold Count. It is known that some species of molds commonly found in wheat and flour will grow slowly at moisture contents of about 14.0 to 14.5% (17). Mold growth might well occur in the samples stored at 14.0% moisture and contribute to the development of fat acidity by increasing the lipase activity of the samples.

Accordingly, mold counts were made on several of the samples after selected periods of storage, employing the method described by Christensen (6).

Influence of Moisture Content, Flour Grade, and Treatment with Chlorine Dioxide on Baking Quality After Various Storage Times

The empirical nature of the experimental baking test and the large number of variables which influence the results make it difficult to secure consistent findings in an experiment of this nature which involves the comparison of baking data obtained at intervals over a 1-year period. Samples of each flour type stored at −18°C. were baked each time tests were conducted on the flours stored at 37.8°C. When the volumes for the controls deviated appreciably from the previous values, the entire bake was repeated. Despite this practice, there were several inconsistencies in the data for some of the samples at successive time intervals; this was particularly evident for the whole wheat flours and is probably associated with the poor properties of the doughs, which made it difficult for the baker to handle them uniformly. Since the symmetry, crumb grain, and texture of the loaves were closely correlated with their volumes, the latter serve as a good over-all measure of baking quality. The various flours were baked with four levels of bromate (0, 1, 2, and 3 mg/100 g) and the loaf volume for that particular dosage of bromate that gave the largest loaf of bread was taken as an index of the potential baking value of the sample under study. The bromate levels at which the largest loaves were obtained increased with decreasing refinement and, in general, decreased as the storage time progressed.

Results with Unbleached Flours. The largest loaf volumes obtained with the unbleached first patent, first and second clear, and whole wheat flours are presented graphically in Figs. 1 and 3, and photographs of the loaves baked from these flours after storage at 3 and 14% moisture for selected intervals are shown in Fig. 2.

For each flour type, the deterioration in baking quality upon storage was greatest in the samples stored at 14% moisture and least in those held at 3 or 6% moisture. Normally, untreated flours improve in baking quality when first stored, but in no case was any improvement noted in this study. However, the storage trials did not begin until 3 months after the samples were milled. Although they were held at −18°C. during most of this period, considerable aging of the samples doubtless occurred during their adjustment to the different

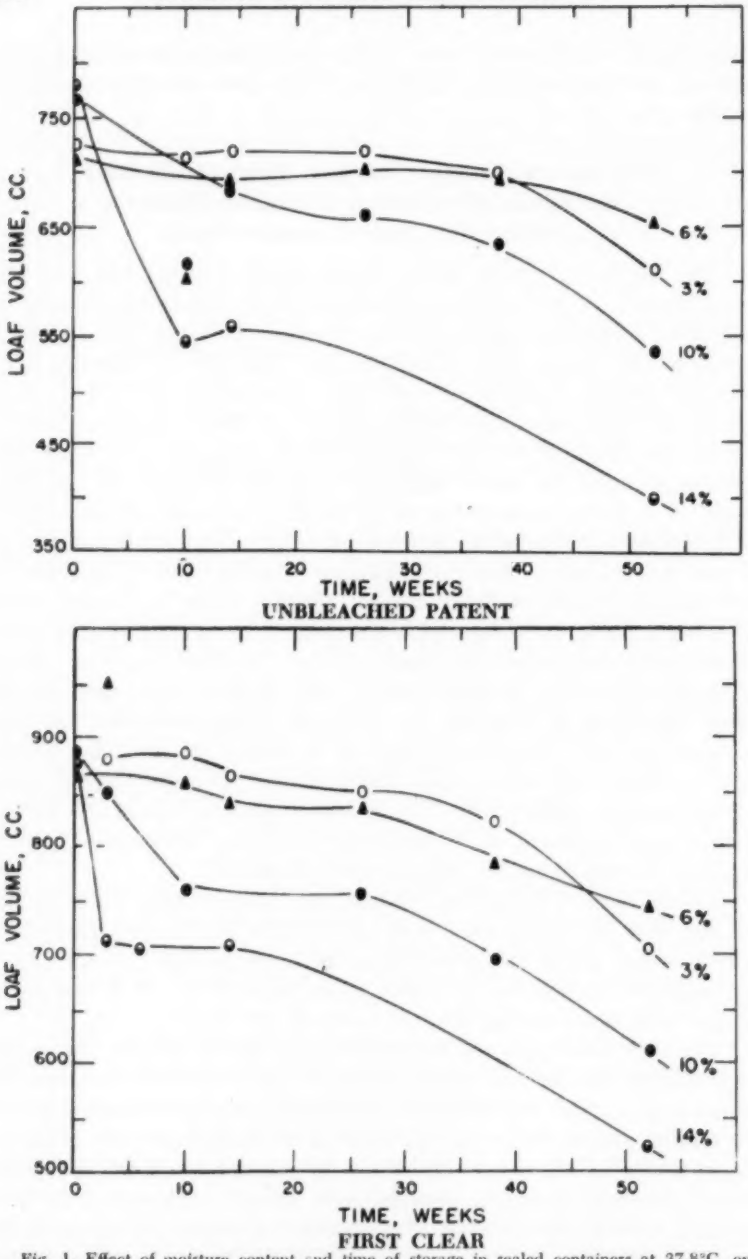

Fig. 1. Effect of moisture content and time of storage in sealed containers at 37.8°C. on the loaf volume of unbleached patent and first clear flours. The volumes represent the maximum values obtained for each sample when baked with appropriate bromate levels.

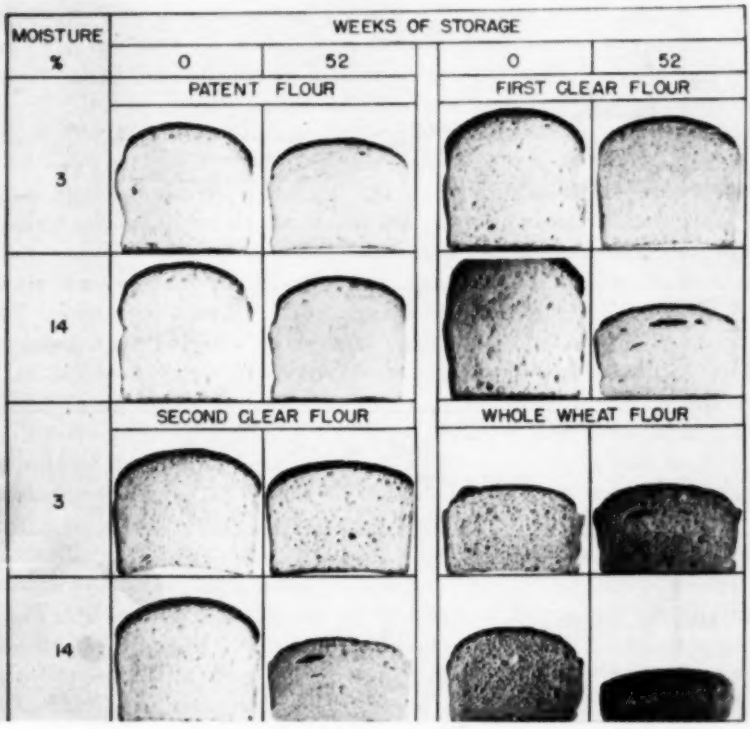

Fig. 2. Photographs of bread baked from unbleached patent, first clear, second clear, and whole wheat flours showing the effect of storage at 3 and 14% moisture in sealed containers for 1 year at 37.8°C.

moisture levels. Also, maturation of the flours was very rapid at 37.8° C. and the flours may well have passed through an improvement phase before the first tests were made.

In general, the breadmaking value of the less highly refined flours fell off more sharply than it did with those which were more highly purified. Thus, at any given moisture content, the short patent flour was the most stable and whole wheat flour the least stable. During 38 weeks' storage at 3 and 6% moisture the loaf volumes of the patent flour remained practically unchanged (Fig. 1 and 2). At 10% moisture there was a gradual decrease in loaf volume during storage, whereas at 14% moisture marked deterioration occurred during the first 10 weeks.

Similar trends were observed in the volumes for the first clear flour, although the rate and extent of deterioration were greater than

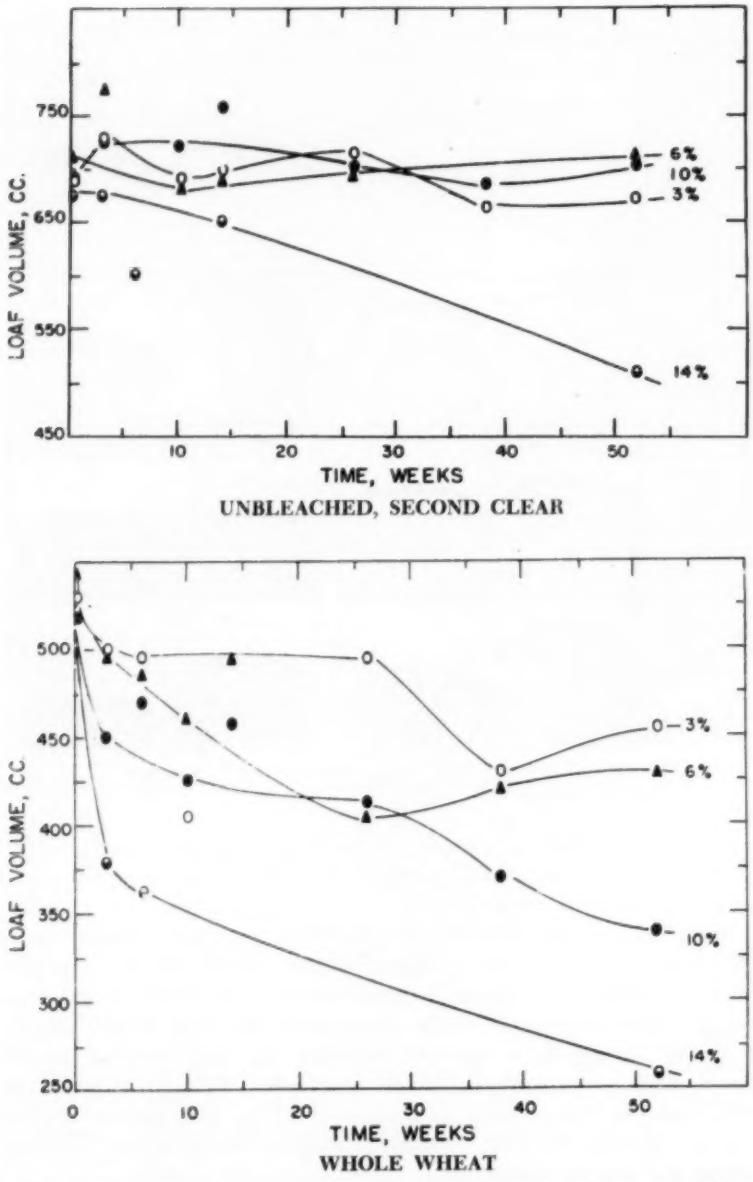

Fig. 3. Effect of moisture content and time of storage in sealed containers at 37.8°C. on the loaf volume of unbleached second clear and whole wheat flours. The volumes represent the maximum values obtained for each sample when baked with appropriate bromate levels.

with patent flour. Second clear flour, on the other hand, showed surprisingly little change in loaf volume at 3, 6, and 10% moisture, and at 14% moisture its stability in breadmaking value compared favorably with that of first clear flour (Figs. 2 and 3).

The baking quality of the whole wheat flour fell off quite rapidly at 10 and 14% moisture; at 14% moisture a very marked decrease had occurred during only 3 weeks' storage (Figs. 2 and 3). Baking data for long patent (patent + first clear) and straight grade flours (patent + first and second clear) at the various moisture levels showed that their breadmaking values decreased somewhat more rapidly upon storage than would have been predicted from the results for the component flours. At 10% moisture their quality began to deteriorate after 26 weeks of storage. With both grades of flour, significant decreases in volume were observed after 38 weeks' storage at 3 and 6% moisture.

Effect of Treatment with Chlorine Dioxide. The changes in loaf volume of the various flours with and without treatment with chlorine dioxide are shown in Table II. Although there are some inconsistencies, it is clear that this type of artificial maturation has an unfavorable effect upon the baking quality of flours subjected to prolonged storage at high temperatures.

It is of interest that during the first part of the storage period, the breadmaking value of the various flours held at 3% moisture was superior to that of those stored at 6% moisture. However, as storage progressed, this superiority became less evident, and after 52 weeks the loaf volumes obtained for the patent, first clear, and second clear flours stored at 6% moisture were the largest. These limited data, when considered in the light of the organoleptic tests for oxidative rancidity to be presented later, lend support to the view that extremely low moistures favor oxidative changes which have a deleterious effect on baking quality.

Effect of Antioxidants, Storage under Nitrogen, and Partial Heat Treatment on the Stability of Whole Wheat Flour. Baking tests on the samples of unbleached and bleached whole wheat flour which had been sprayed with 0.01% by weight of AMIF 72 and monoisopropyl citrate failed to reveal that these treatments had any significant influence on the rates of change in the baking qualities of these flours upon storage at the four moisture levels. Likewise, baking tests with the sample of whole wheat flour stored at 14% moisture after being evacuated and flushed with nitrogen several times did not show that this treatment retarded the deterioration in breadmaking value.

TABLE II

INFLUENCE OF FLOUR GRADE AND TREATMENT WITH CHLORINE DIOXIDE ON THE CHANGES IN OPTIMUM LOAF VOLUME UPON STORAGE AT 37.8°C.

Flour Grade	Storage Moisture	Untreated			Treated		
		Storage Time, Weeks			Storage Time, Weeks		
		14	26	52	14	26	52
	%	cc.	cc.	cc.	cc.	cc.	cc.
Patent	3	− 5	− 5	−115	− 45	−100	−200
	6	− 15	− 10	− 60	0	− 15	−215
First clear	3	− 35	− 50	−195	− 5	−520	−555
	6	− 60	− 65	−160	+ 25	−375	−480
	10	...	−145	−190	−105	−375	−470
	14	−190	...	−380	−170	...	−515
Second clear	3	+ 5	+ 15	− 25	+ 5	− 15	− 40
	6	− 10	− 5	+ 15	− 25	−105	− 70
	10	...	0	+ 5	...	− 80	−115
	14	− 50	...	−190	− 5	...	−305
Long patent	3	0	− 55	−100	− 60	− 95	−160
	6	+ 10	− 50	− 80	0	− 45	− 75
	10	− 85	−125	−245	− 20	−125	−210
	14	...	...	−310	...	...	−290
Straight grade	3	− 20	− 65	−160	− 20	−115	− 70
	6	+ 50	+ 35	− 75	+ 55	−100	− 50
	10	− 60	−115	−220	− 90	−115	−245
	14	...	...	−265	...	...	−280
Whole wheat	3	− 30	− 35	− 75	− 40	−100	− 95
	6	− 50	−145	−115	− 55	...	−140
	10	− 70	−105	−180	−135	−190	−245
	14	−140	...	−275	−190	...	−320

Recently Cryns *et al.* (8) have shown that the rapid deterioration of whole wheat flour in storage, which has hitherto limited its use by the armed forces, may be partially circumvented by heat-treatment of the feed by-products which are removed in milling white flour. The dried and ground mixture can then be stored in airtight containers and added to white flour as required to prepare a whole wheat product for baking. Storage tests at 37.8°C. over 1 year showed that the "wheat base" suffered no change in odor, flavor, or baking quality.

It was, therefore, of interest to determine whether a mixture of heat-processed feed products with white flour would be stable upon storage. The results, to be presented later (Table V), showed that the baking quality of the mixture fell off very markedly on storage.

Influence of Moisture Content, Flour Grade, and Treatment with Chlorine Dioxide on Changes in Extensograph Properties, Gas Production, and Retention upon Storage at 37.8°C.

Extensograms. The results of extensograph tests on the various

TABLE III

EFFECT OF MOISTURE CONTENT, FLOUR GRADE, AND TREATMENT WITH CHLORINE DIOXIDE ON CHANGES IN EXTENSOGRAM PATTERNS WITH STORAGE

Flour Grade	Treatment	Moisture	Storage Time: 0 weeks Height	0 weeks Length	6 weeks Height	6 weeks Length	14 weeks Height	14 weeks Length
		%	*B.U.*	*B.U.*	*B.U.*	*B.U.*	*B.U.*	*B.U.*
Patent	None	3	430	12	720	10	...	...
		6	450	13	820	10	450	20
		10	480	14	670	14	410	20
		14	300	21	710	14	480	32
Patent	Chlorine dioxide	3	700	10	700	11	...	...
		6	600	15	780	9	...	...
		10	500	18	800	9	...	...
		14	510	14	880	9	...	...
First clear	None	3	670	20	800	12	580	17
		6	...	...	990	10	570	17
		10	...	...	1000	13	690	18
		14	...	...	1000+	12	910	13
First clear	Chlorine dioxide	3	800	14	720	11	810	14
		6	720	14	840	10	850	11
		10	750	14	950	10	960	11
		14	730	12	930	9	750	12
Second clear	None	3	460	25	540	30	...	...
		6	420	25	530	29	320	29
		10	300	30	570	23	440	28
		14	330	30	...	...	...	...
Second clear	Chlorine dioxide	3	770	17	730	18	540	20
		6	670	20	830	14	490	22
		10	610	25	860	12	620	18
		14	...	...	740	12	...	...
Straight flour	None	3	850	9	770	14	660	9
		6	840	10	700	13	500	16
		10	700	10	550	16	...	...
		14	760	9	780	13	790	11
Straight flour	Chlorine dioxide	3	750	10	820	12	670	12
		6	790	10	940	11	590	14
		10	780	12	800	12	630	13
		14	810	12	860	11	...	...

grades of white flour, with and without artificial maturation, after storage at 37.8°C. for 0, 6, and 14 weeks are summarized in Table III.

Within each grade of flour, the changes in the extensogram patterns of the unbleached flour upon storage were greater at 14% than at the lower moistures employed. Initially, most of the flours gave curves of the lowest height and greatest length at 14.0% moisture; this probably was caused by some maturation of the low-moisture samples during the drying operations preceding storage. The heights of the extensograms for the unbleached patent, first clear, second clear, and long patent flours increased during the first 4 weeks of storage at all moisture levels and decreased again after 14 weeks' storage at 14, 10, and 6% moisture. Similar trends were observed for the changes in area enveloped by the curves.

Artificial maturation had a marked influence on the extensogram

patterns of the flours. Initially, the patterns for the treated flours were higher than those for the untreated ones, and upon storage for 6 weeks the patterns for the latter increased in height and area, whereas those for the artificially matured flours changed very little in these respects.

In general, natural aging and artificial maturation had similar effects on the extensogram patterns, namely an increase in height, decrease in length, and increase in area. Their effects were supplementary until maximal values were attained, after which additional oxidation resulted in curves of lower height and area.

Gas Production and Retention. The tests with the Chopin Zymotachygraphe were limited to unbleached patent and whole wheat flours after storage for 0, 26, and 52 weeks at 14% moisture and 37.8°C. The gas production and retention values for 6 hours at 30°C. are recorded in Table IV, together with the loaf volumes obtained in the baking tests with optimum bromate levels.

TABLE IV

EFFECT OF STORAGE OF UNBLEACHED PATENT AND WHOLE WHEAT FLOURS AT 14% MOISTURE AND 37.8°C. UPON GAS PRODUCTION AND RETENTION AS MEASURED IN THE ZYMOTACHYGRAPHE

Flour	Storage Time	Loaf Volume[b]	Zymotachygraphe Values[a]		
			Gas Production	Gas Retention	Time of Maximum Retention
	weeks	*cc.*	*ml.*	*ml.*	*min.*
Patent	0	700	1830	1470	140
	26	560	2130	1480	90
	52	440	2160	1470	120
Whole wheat	0	590	2170	1430	80
	26	440	1830	1305	60
	52	235	1710	1212	50

[a] Tests were carried out for 6 hours at 30°C.
[b] Obtained with optimum bromate.

The data show that the gas production of the patent flour dough increased, while that of the whole wheat flour dough decreased as a result of storage. The gas retention of the patent flour dough did not change, while that of the whole wheat flour decreased with storage. In the doughs from both flours, loss of gas from the dough occurred sooner with the flours stored at 37.8°C. than for the controls. There is no clear-cut relation between the zymotachygraphe data and the loaf volume of these flours.

Factors Influencing the Development of Objectionable Odors During Storage at 37.8°C.

Although only four observers participated in the organoleptic tests, their evaluations were in surprisingly good agreement and the mean values proved to be sensitive indices of biochemical changes in the samples.

White Flours. With all grades of white flour the development of objectionable odors became more pronounced as the moisture content was increased from 6 to 10 to 14%; however, several of the flours stored at 3% moisture were rated lower than those which contained 6% moisture. For example, patent flour stored at 3, 6, 10, and 14% moisture developed undesirable odors after 26, 38, 10, and 3 weeks' storage, respectively. Very similar trends were observed for the other grades of flour. In agreement with the baking results presented in a previous section, second clear flour was surprisingly stable; in fact, at 6 and 10% moisture it was the most stable of all the flours.

As anticipated, the majority of the flours which had been treated with chlorine dioxide developed objectionable odors sooner than the corresponding unbleached samples stored under similar conditions. This effect was observed with first clear flour (3, 6, and 10% moisture), second clear flour (6, 10, and 14% moisture), long patent flour (3 and 6% moisture), and straight grade flour (10 and 14% moisture). The differences between the bleached and unbleached samples became less obvious as storage progressed, and after 52 weeks no significant differences in odor intensity were detected.

Feed Products. Red dog, brown middlings, bran, and germ developed objectionable odors more rapidly and extensively than the various grades of flour. Even at 3% moisture undesirable odors were detected after only a few weeks' storage (bran in 3 weeks, brown middlings and germ in 6 weeks, and red dog in 14 weeks).

Whole Wheat Flour. In conformity with the results obtained on white flours, the development of objectionable odors in whole wheat flour was more rapid and extensive as the moisture content during storage was increased; in several instances, however, undesirable odors were detected in samples held at 3% moisture before they were observed in those stored at 6%. Objectionable odors were detected in the samples treated with chlorine dioxide sooner than in the unbleached samples.

The influence of the two antioxidants, AMIF 72 and monoisopropyl citrate, on stability were tested by adding 0.01% to bleached whole wheat flours. Only with the addition of AMIF 72 to flour stored

at 3% moisture was there any effect detected: in this instance the development of an abnormal odor was somewhat delayed. A larger and more highly trained panel might possibly have succeeded in detecting other differences.

Heat-treatment of the feed components before blending with the flour fractions to produce whole wheat flour did not prevent the development of undesirable odors upon storage (Table V). Actually,

TABLE V

EFFECT OF HEAT-PROCESSING FEED STREAMS USED IN PREPARING WHOLE WHEAT FLOUR ON ITS STABILITY AT 37.8°C.

Property	Treatment of Feed Streams	Storage Time in Weeks				
		0	3	6	10	52
	Stored at 3% moisture					
Loaf volume, cc.	None	520	480	470	450	425
	Heated	425	340	365	405	300
Fat acidity	None	85	82	73	80	66
	Heated	66	...	62	67	67
Organoleptic score	None	1	1	1	1	4
	Heated	1	1	2	2	2
	Stored at 10% moisture					
Loaf volume	None	575	440	465	420	330
	Heated	470	395	455	400	290
Fat acidity	None	98	232	302	326	...
	Heated	74	78	90	98	170
Organoleptic score	None	1	1	2	2	...
	Heated	1	1	2	2	3
	Stored at 14% moisture					
Loaf volume	None	570	380	300	...	250
	Heated	460	350	340	310	220
Fat acidity	None	142	298	346	363	...
	Heated	96	92	95	107	159
Organoleptic score	None	1	1	2	3	...
	Heated	1	1	2	...	3

the whole wheat flour prepared with the heat-treated concentrate had an undesirable odor after only 6 weeks of storage at 3% moisture, in contrast with the odor of the unheated whole wheat flour which was normal until 38 weeks of storage. It would appear that heat-treatment of the feed streams tends to favor the development of oxidative rancidity in whole wheat flour blends, stored at low moisture contents,

presumably owing to the destruction of such natural antioxidants as tocopherols which are present in the wheat.

Storage of the whole wheat flour under nitrogen did not delay the development of objectionable odors. Small percentages of oxygen (in most instances below 1.0%) were present in the gas removed from these samples.

Influence of Various Factors on Chemical Changes in the Flour Lipids During Storage

Fat Acidity. The development of fat acidity during storage of the unbleached patent, first clear, second clear, and whole wheat flours at the four moisture levels is shown graphically in Figs. 4 and 5 respectively. In Fig. 6, the changes in the fat acidity of these flours, brown middlings, and wheat germ during storage at 14% moisture are plotted on the same scale.

The development of fat acidity was related to the degree of refinement and the moisture contents at which the various products were stored. At 3% moisture, there was virtually no change in fat acidity throughout the 52-week storage period, whereas at 14% it developed so rapidly that in many instances the free fatty acids, expressed as oleic acid, exceeded 50% of the total lipid fraction. A similar situation existed in the second clear flour and whole wheat flours after 26 and 38 weeks' storage respectively at 10% moisture. Limitations in available substrate are reflected by the leveling off in the development of fat acidity in unbleached first clear, second clear, and whole wheat flours stored at 14% moisture (Figs. 4 and 5) and in unbleached second clear and whole wheat flours at 10% moisture (Fig. 5). It is significant that the development of fat acidity in the various materials (Fig. 6) is in the same order as their lipid contents (Table I).

In general, the fat acidities of the bleached flours were in good agreement with those for the unbleached samples. Heat treatment of the feed components before their admixture with white flour fractions to prepare whole wheat flour markedly decreased the development of free fatty acids (Table V). However, the addition of 0.01% of AMIF 72 and monoisopropyl citrate to whole wheat flour had no effect.

Relation Between Fat Acidity and Loaf Volume. A comparison of the curves for changes in loaf volume upon storage (Figs. 1 and 3) with those for fat acidity (Figs. 4 and 5) indicate an inverse relation between these variables. Accordingly, correlation coefficients were computed. Since artificial maturation had no influence on fat acidity and the loaf volumes utilized as a measure of potential baking value

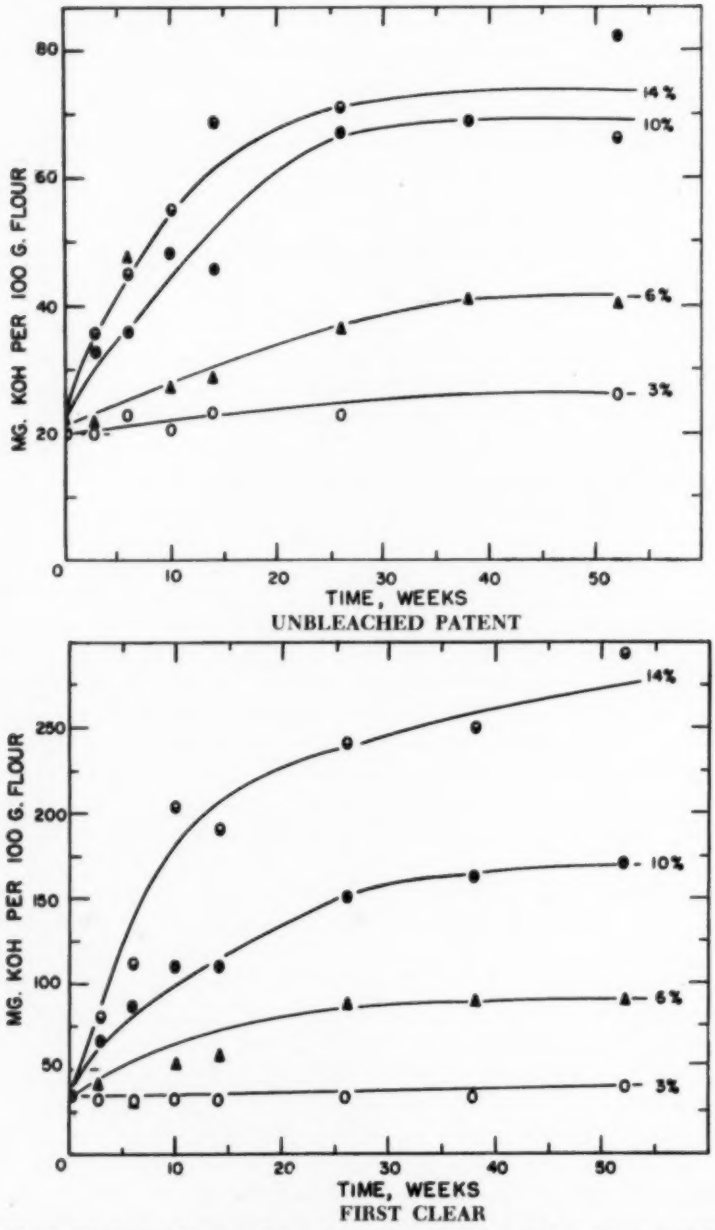

Fig. 4. Effect of moisture content and time of storage in sealed containers at 37.8°C. on the fat acidity of unbleached patent and first clear flours.

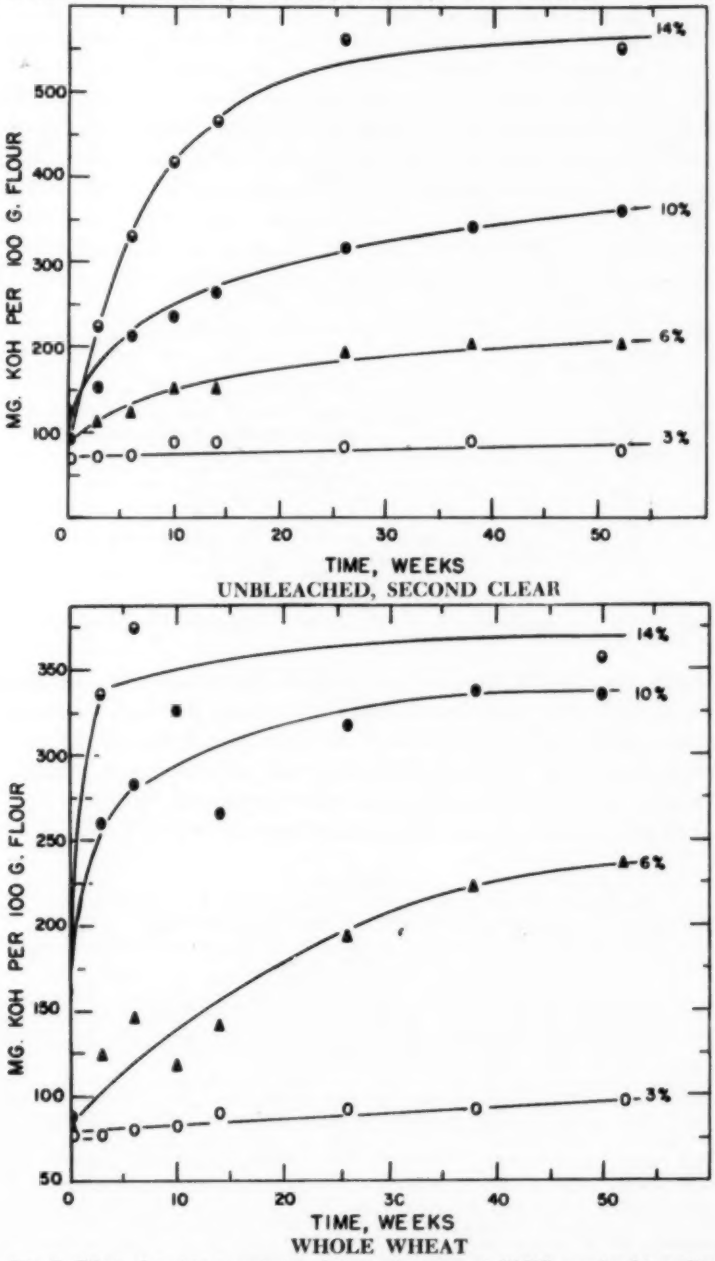

Fig. 5. Effect of moisture content and time of storage at 37.8°C. on the fat acidity of unbleached second clear and whole wheat flours.

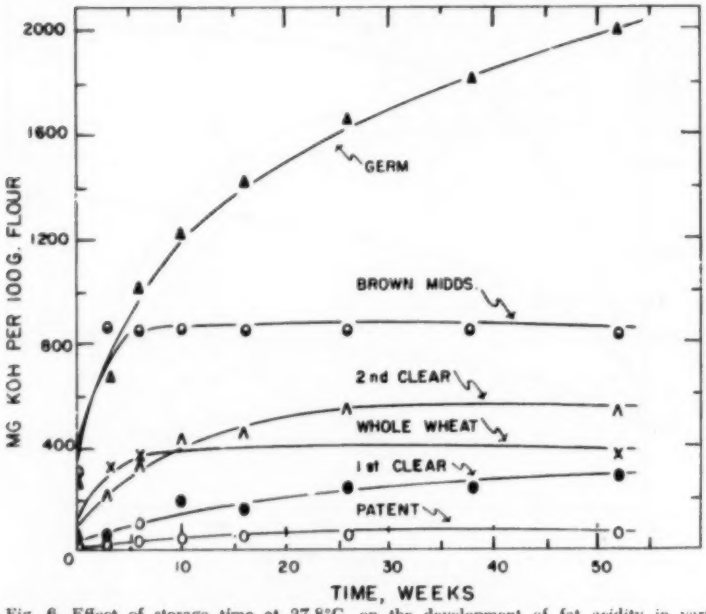

Fig. 6. Effect of storage time at 37.8°C. on the development of fat acidity in various wheat products containing 14% moisture.

were the maxima obtained for each flour without respect to the dosage of bromate, it was considered justifiable to pool the data for the corresponding treated and untreated samples. The flours stored at 3% moisture were not included, since they did not show any changes in fat acidity nor loaf volume (except after prolonged storage).

The results of the statistical analyses summarized in Table VI show

TABLE VI
STATISTICAL CONSTANTS FOR LOAF VOLUME AND FAT ACIDITY OF FLOURS STORED AT 6, 10, AND 14% MOISTURE

Flour	No. of Samples	Mean		Standard Deviation		Correlation Coefficient	Regression Coefficient[b]	Standard Error of Prediction[c]
		Loaf Volume	Fat Acidity[a]	Loaf Volume	Fat Acidity			
		cc.	*mg.*	*cc.*	*mg.*			*cc.*
Patent	32	643	48.6	103	22.7	—0.77	—3.50	66
First clear	37	687	91.8	178	55.8	—0.51	—1.64	153
Second clear	37	684	244.4	74	128.4	—0.62	—0.35	58
Whole wheat	77	444	234.3	77	86.6	—0.86	—0.76	39

[a] Fat acidity is expressed as mg KOH/100 g flour (14.0% moisture basis).
[b] Regression of loaf volume on fat acidity.
[c] Standard error of prediction of loaf volume from fat acidity.

that there is a significant negative correlation between the loaf volume of the respective grades of flour and fat acidity. Considering that the data for bleached and unbleached flours were combined, that relatively large errors likely occurred in baking tests conducted at different time intervals over a one-year period, and also that oxidative changes influence the breadmaking value of flours, the magnitude of the correlations was surprisingly high.

A scattergram showing the relationship between the loaf volume of whole wheat flour and fat acidities is given in Fig. 7. These results

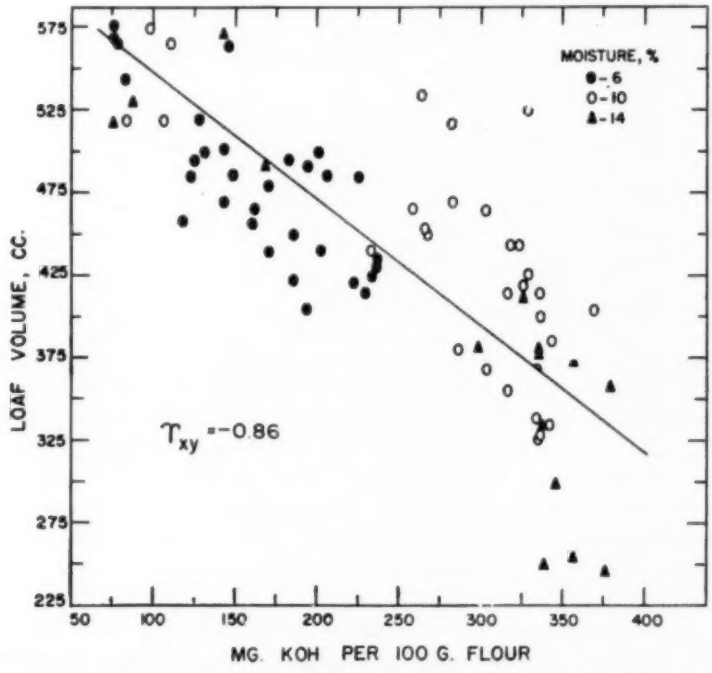

Fig. 7. Scattergram showing the relation between loaf volume and fat acidity of whole wheat flours. The loaf volumes represent the maximum values obtained for each sample with appropriate bromate levels. Data for unbleached and bleached flours and also for samples treated with two antioxidants and stored under nitrogen at 37.8°C. are included.

suggest that the increases which occur in fat acidity of wheat flours may serve as a fairly reliable index of the over-all changes taking place upon storage which influence baking quality.

To secure a direct measure of the influence of changes in the lipids on breadmaking value, samples of straight grade and whole wheat flour stored at —18°C. and for several months at 37.8°C. were ex-

tracted with diethyl ether for 8 hours by means of a water bath maintained at 50°C. The extracted flours were spread in thin layers and allowed to stand overnight, then heated at 37.8°C. for 1 hour to remove residual traces of ether. The extracted lipids were recovered in the usual manner and added back to the respective flours. In addition, the fresh lipids (obtained from the flours stored at —18°C.) were added to the extracted spoiled flours (original flours stored at 37.8°C.), and *vice versa*. Baking tests were conducted at bromate levels of 0, 1, 2, and 3 mg. per 100 g. flour, and the maximum volumes irrespective of bromate level are recorded below:

	Loaf Volumes	
	Straight Grade	*Whole Wheat*
	cc.	*cc.*
Fresh flour	770	640
Extracted fresh flour plus fresh lipids	695	620
Extracted fresh flour plus spoiled lipids	625	565
Spoiled flour	560	220
Extracted spoiled flour plus fresh lipids	675	335
Extracted spoiled flour plus spoiled lipids	525	240

These data clearly show that the biochemical changes which occur in the lipids of deteriorated flour have a marked influence on breadmaking value. However, the replacement of the deteriorated lipids with those from sound flour did not restore the baking quality of either straight grade or whole wheat flour to its original value. Although extraction of the lipids is not complete with ethyl ether, the data indicate that biochemical changes in the nonlipid constituents during storage are of great significance in the instance of whole wheat flour.

Oxidative Changes in the Lipids. The results of the peroxide and monocarbonyl determinations on the lipids extracted from the flours after various periods of storage were disappointing. As shown in Table VII, these measures proved less sensitive than the results of organoleptic tests on the flours. The iodometric method of Wheeler (24) gave low and inconsistent values and its use was discontinued early in the trials. The data given here, obtained by a modification of the ferric thiocyanate procedure of Hills and Thiel (11), were more satisfactory. The monocarbonyl values by the method of Pool and Klose (18) were not closely related to the extent of rancidity as measured by smelling the samples[6].

TABLE VII

COMPARISON OF ORGANOLEPTIC SCORES FOR BLEACHED FLOURS OF VARYING PURITY WITH PEROXIDE AND MONOCARBONYL VALUES ON THE LIPIDS EXTRACTED AFTER VARIOUS STORAGE TIMES AT 3% MOISTURE AND 37.8°C.

Weeks of Storage	Organoleptic Test (1–6)	Peroxide Value ($\mu e/kg$)	Monocarbonyl Value (μ moles/kg)
	Patent flour		
0	1	20	0.5
26	2	60	.0
38	3	110	.0
52	4	400	6.5
	First clear flour		
0	1	30	.5
26	2	60	.0
38	2	60	5.5
52	3	410	17.5
	Whole wheat flour		
0	1	00	.0
26	1	00	.0
38	3	60	.0
52	4	120	0.0

Influence of Flour Grade, Artificial Maturation, and Storage at Various Moisture Contents on Sulfhydryl Content

The results of sulfhydryl determinations on bleached and unbleached patent, first clear, and second clear flours after storage at different moisture contents are summarized in Table VIII.

The sulfhydryl contents of the flours increased with decreasing refinement but were not appreciably influenced by treatment with chlorine dioxide. At 3% moisture, there was no significant change in the sulfhydryl values upon storage, but decreases were found at 6, 10, and 14% moisture, the largest being at 14%. The changes noted upon storage were the greatest with the second clear flour.

Effect of Moisture Content and Storage Time on the Loss in Thiamine from Different Wheat Products

The results of thiamine assays in Table IX show that the moisture content at which the wheat products were stored has a marked influence on the extent to which thiamine is destroyed. Upon storage at 37.8°C. at 14% moisture, some destruction was evident after only 3 weeks' storage; after 52 weeks, the red dog and wheat germ streams

[8]Since this work was completed, preliminary tests with the procedure of Neumer and Dugan (Neumer, J. F., and Dugan L. R., Jr. A new method for measuring the development of rancidity. Food Technol. 7: 191-194. 1953) revealed marked increases in the carbonyl values of lipids when they undergo oxidation.

TABLE VIII

CHANGES IN SULFHYDRYL CONTENT OF FLOURS WITH STORAGE TIME AND MOISTURE CONTENT

Flour	Moisture Content	Storage Time in Weeks			
		0	6	14	38
		–SH Equivalents × 10^6 Per G. Flour			
	%				
Patent flour	3	3.2	3.1	3.3	3.0
	6	3.1	3.1	3.1	3.1
	10	3.2	3.4	3.1	2.8
	14	3.2	3.3	2.9	2.5
Patent flour, bleached	3	3.3	3.1	3.3	3.0
	6	3.1	3.2	3.3	2.9
	10	3.0	3.2	3.1	2.8
	14	3.0	3.1	2.9	2.7
First clear	3	3.8	3.9	3.4	3.9
	6	3.8	3.9	3.4	3.7
	10	3.6	3.7	3.8	3.3
	14	3.6	3.5	3.3	3.1
First clear, bleached	3	3.4	3.5	3.6	3.4
	6	3.2	3.4	3.4	3.1
	10	3.3	3.4	3.3	3.1
	14	3.1	3.4	3.2	3.1
Second clear	3	4.5	5.2	4.8	4.3
	6	4.4	5.0	4.7	4.1
	10	4.4	5.2	4.8	3.9
	14	4.5	4.1	3.5	3.4
Second clear, bleached	3	4.6	4.8	4.8	4.3
	6	4.5	4.9	4.6	4.2
	10	4.2	4.2	4.3	3.6
	14	4.2	4.1	3.8	3.2

had lost about 80% of their original thiamine. The losses were greatly reduced in the samples stored at lower moistures; in fact, there were no consistent changes in the thiamine contents of whole wheat flours stored at moisture contents of 3, 6, and 10%.

Effect of Moisture Content and Storage Time on the Mold Count of Different Wheat Products

Mold spore counts on samples of the various flours and wheat products were lower than in samples of commercial flours tested by Christensen and Cohen (7). The counts for most of the samples were lower at the end of the trial than at earlier periods, even in the samples at 14% moisture, indicating that molds were not a factor of any significance in the marked deterioration which occurred in the samples stored at this moisture level.

TABLE IX

EFFECT OF MOISTURE CONTENT AND STORAGE TIME AT 37.8°C. ON THE THIAMINE CONTENT OF DIFFERENT WHEAT PRODUCTS[a]

Wheat Product	Moisture Content	Thiamine Content[b] during Storage Period (weeks)			
		0	3	38	52
	%	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*
Whole wheat flour	3	2.6	2.7	2.5	2.8
	6	2.5	2.5	2.4	2.5
	10	2.6	2.6	2.1	2.3
	14	2.4	2.4	...	1.3
Whole wheat flour, bleached	3	2.7	2.7		2.5
	6	2.6	2.5	2.4	2.5
	10	2.5	2.6	2.1	...
	14	2.5	2.3	1.6	...
Whole wheat flour, bleached, plus AMIF 72	3	2.8	2.7	2.4	2.6
	6	2.5	2.5	2.3	2.6
	10	2.4	2.5	2.0	2.4
	14	2.6	2.3	1.6	1.1
Whole wheat flour, heat-treated	3	2.5	2.4	2.2	2.5
	10	2.4	2.2	2.0	2.0
	14	2.6	2.2	1.5	1.4
Red dog	3	17.8	...	17.1	16.6
	6	17.9	...	16.8	18.0
	10	17.8	17.8	12.4	9.9
	14	19.3	18.1	7.3	3.6
Wheat germ	3	10.1	11.3	8.8	10.1
	6	11.2	11.2	8.8	9.4
	10	10.8	10.4	8.5	8.0
	14	...	10.5	4.8	2.1

[a] These analyses were carried out by the Quality Control Laboratory of Pillsbury Mills, Inc., Minneapolis, Minnesota.
[b] Dry basis.

Discussion

These experiments with wheat flours stored in airtight containers at 37.8°C. clearly demonstrate that the stability of such bread flours depends upon their degree of refinement, the artificial maturation with chlorine dioxide which they received, and upon the moisture content at which they were stored. Flours destined for prolonged storage under severe conditions should be of the highest purity which is consistent with cost and should not be treated with oxidizing improvers. When treatment with chlorine dioxide is superimposed upon natural aging, "overtreatment" eventually results, after which the baking quality falls off quite rapidly. Lowering the moisture content below normal levels will markedly increase the stability of wheat flours in storage. While changes in the lipids were the most striking of all the chemical properties which were investigated, definite evidence

was obtained that the deterioration in breadmaking value also involves nonlipid constituents, especially in the case of whole wheat flour. Replacement of the bulk of the lipids of deteriorated whole wheat flour with those from sound flour did not fully restore its baking quality. The fact that the increases in fat acidity were significantly correlated with the losses in breadmaking value suggests that the fat acidity within any one flour grade may prove to be a useful index of the over-all changes involved in deterioration. While measurements of peroxide and monocarbonyl value failed to reveal that any extensive oxidative changes took place, the marked development of rancid odors as storage progressed leads to the conclusion that oxidation of the lipids was a factor in deterioration. Improved methods for measuring the changes as well as for estimating sulfhydryl compounds would be valuable in studies of this nature.

Acknowledgments

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DYE-BINDING CAPACITIES OF WHEAT FLOUR PROTEIN FRACTIONS[1]

DOYLE C. UDY[2]

ABSTRACT

Acid and basic groups of protein molecules have been measured quantitatively on fractions of wheat protein by dye-binding techniques. These fractions were essentially soluble proteins, gluten proteins, a combination of these two, and proteins nondispersible in dilute acetic acid.

The acid binding groups exhibit considerable constancy in their dye-binding capacity within a given fraction for all varieties examined. The highest binding capacities were obtained with the soluble protein fraction, and the lowest with the most insoluble fraction. The base binding groups followed this same pattern with the exceptions that the soluble fraction gave the lowest binding capacities for some varieties, and differences in binding capacity between fractions were much less marked.

The substantial varietal difference in viscosity behavior of dispersed gluten proteins arising from electrostatic interactions is not likely associated with the primary valence forces operative in the dye-binding reaction. The electrostatic differences probably originate with weaker molecular forces.

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[2] Assistant Chemist, Western Wheat Quality Laboratory, Department of Agricultural Chemistry, Agricultural Experiment Stations, Pullman, Washington.

Some evidence has been reported for relating certain differences in wheat glutens to the electrostatic properties they inherently possess (6). Certain groups on protein molecules which can dissociate and form ions, or those sites that are of sufficient polarity to accommodate an ionized molecule, should be receptive to a binding reaction with an ion of opposite charge. At low and high pH the basic and acidic groups on a protein molecule are charged positively and negatively, respectively. A convenient method for measuring the acidic or basic groups on proteins using an insoluble protein-dye complex has been developed by Fraenkel-Conrat and Cooper (1).

This paper presents the dye-binding capacities of four protein fractions from each of several varieties of wheat by the method cited above with some minor modifications. These measurements enabled a comparison of the number of primary charges on a given protein fraction between the varieties examined, and also between the protein fractions. The results obtained give further information on the nature of the electrostatic forces which are responsible for differences in viscosity for dispersions of wheat glutens.

Materials and Methods

Preparation of Protein Fractions. Proteins from the flour of several varieties of wheat have been divided into four general fractions, A, B, C, and D. Fraction A includes the proteins dispersible in 0.05% phosphate buffer[3] when flour is dispersed in a Waring Blendor for 3 minutes and then centrifuged at 3000 r.p.m. to remove the non-dispersed solids. The B fraction includes proteins not dispersed in the phosphate buffer solution but dispersed in 0.03 *N* acetic acid. This is essentially the fraction usually referred to as gluten. Fraction C is the sum of fractions A and B and was obtained by dispersing flour directly in 0.03 *N* acetic acid. Preliminary extractions of protein from flour using acetic acid ranging from 0.03 to 1 *N* showed that equal quantities of protein were extracted in all cases. The protein not dispersed by buffer or acetic acid was fraction D. The protein content in each fraction was determined by Keys' (2) micro-Kjeldahl method.

Measurement of Bound Dye. Orange G, a water-soluble sulfonic acid dye of 93% dye content, was obtained from Allied Chemical and Dye Corporation. It was used without further purification in a 0.1% solution buffered to pH 2.2 using McIlvaines' buffering constituents in the specified ratio. Another water-soluble dye, safranin O, of 95%

[3] The buffer is a 0.05% aqueous solution of an equal weight percent mixture of $Na_2HPO_4 \cdot 12H_2O$ and $NaH_2PO_4 \cdot H_2O$, having a pH around 7.

dye content was obtained from the same source. This dye, a quaternary ammonium salt, was also used without further purification in a 0.2% solution buffered to pH 11.5 using 17.90 g. dibasic sodium phosphate dodecahydrate and 1.12 g. potassium hydroxide per liter.

Approximately 20-40 mg. of protein, either solid or in dispersion, was reacted with 25 ml. of 0.1% orange G or 0.2% safranin O dye solution in a 250 ml. centrifuge bottle. Protein fractions dispersed in dilute acetic acid were neutralized in the centrifuge bottle with an equivalent amount of 0.3 *N* potassium hydroxide before adding the dye solution. Fraction A was heated at the boiling point for 3 to 4 minutes in the neutral buffer solution before adding the dye solution. After about 15 hours the contents were centrifuged at 3000 r.p.m. for 10 minutes and the supernatant decanted from the precipitated protein-dye complex.

Another procedure used with fraction A (Table II column 1) involved placing the undenatured protein dispersion in a cellophane tubing and equilibrating with dye solution in a glass stoppered tube. The small amount of dye preferentially bound by the tubing had to be determined and accounted for in subsequent calculations.

The clear supernatant was diluted 50 fold and its optical density measured with a Beckman model DU spectrophotometer at a wave length of 472 mμ for orange G dye solutions and 452 mμ for solutions of safranin O dye. Stock solutions of these dyes were diluted 100 fold before measuring their optical densities. The diluent used for the safranin O dye solutions was 0.1 *N* acetic acid which prevented the dye from adsorbing to the glass surfaces. All solutions conformed to Beer's law. Binding capacity values were all calculated in terms of acid or base equivalents per gram of protein. The equivalent weights used for orange G and safranin O dye were 226 and 355, respectively. The dye content and moisture of the dye preparations were accounted for in the calculations.

Results and Discussion

Binding of Safranin O at pH 11.2. Acidic groups dissociating at pH 11.2 include the carboxyl, phenol, and thiol. This pH probably represents the optimum conditions (4) for maximum dissociation without undue damage to the protein molecules. The affinity of safranin O for anionic centers posed several difficulties in measurement of the dye-binding capacities of the several fractions of protein, as will be evident in the following discussion.

Table I summarizes the results of the measurements made on each

fraction with safranin O dye. These values were not nearly so conclusive as those obtained with orange G, yet a definite trend was indicated. Dye-binding by fraction D was not feasible because of the high affinity of safranin O for the large amount of starch present

TABLE I

DYE-BINDING CAPACITIES[a] OF ACIDIC GROUPS
(Safranin O dye)

Variety	Fraction		
	A[b]	B	C
Wasatch	7.1	6.0	6.2
Idaed	5.9	6.3	6.3
Elgin	5.7	6.3	6.1
Golden	7.3	6.0	6.2
Brevor	6.3	6.0	6.4
Federation	5.9	6.0	
Rio	5.7	6.1	

[a] Base equivalents of safranin O dye/g. protein $\times 10^4$.
[b] Heated 3-4 minutes at neutral pH.

(18.4 mg. dye/g. starch). This binding undoubtedly results from interaction of dye with acid groups commonly present in polysaccharides.

Values for fraction A show some irregularity, but this is not too surprising for proteins having been denatured. Several unsuccessful attempts were made to measure the acid groups of this fraction in the native state by using cellophane tubing to contain the protein dispersion while equilibrating with dye solution from the outside. While this modification in the method gave good clear solutions for spectrophotometric measurement which could not be obtained otherwise due to incomplete settling of the dye-protein complex upon centrifuging, another phenomenon was introduced which made it of little value. The obstacle encountered was the high sorption of dye by the cellophane presumably as a result of acid groups present in the cellulose. The sorption varied with the concentration of free dye. Washing, drying, and weighing of the cellophane sacs used helped some, but the results were not considered reliable. This complication was not encountered with orange G, since it was only mildly bound by the cellophane and did not vary with free dye concentration.

Fractions B and C are not significantly different in their dye-binding capacity, which would indicate that fraction A should have approximately the same value. This in turn would suggest that the denaturing process did not affect the capacity of the acidic groups to bind dye nearly so much (if any) as did the same treatment when meas-

uring the basic groups for this same fraction (discussed in the next section).

Binding of Orange G at pH 2.2. Basic groups on the protein molecules acquiring sufficient charge for dissociation at pH 2.2 and consequently the ability to combine with an ion of opposite charge are the guanidyl, imidazole, and amino groups, as indicated by the work of Rawlins and Schmidt (5). Uniformity in the pH condition of the reaction mixtures was accomplished by using a buffered solution of either dye in a ratio of 2.5 ml. of dye solution to 1 ml. of protein dispersion.

The acid equivalents of dye bound by 1 g. of protein for the four fractions of protein described above were obtained from several varieties of wheat and are listed in Table II. It should be emphasized that the different varieties fail to show any significant variations within any given protein fraction. Differences in binding capacity for a given

TABLE II

DYE-BINDING CAPACITIES[a] OF BASIC GROUPS
(Orange G dye)

Variety	Fraction					
	A	A[b]	B	B[c]	C	D
Wasatch	8.0	6.1	4.6		5.1	
Idaed	8.0	5.9	4.6		5.1	
Rio	7.8	6.0	4.6		4.9	3.1
Brevor	8.0	5.9	4.5	6.6	4.9	
Golden	7.8	5.9	4.5	6.5	5.0	2.4
Elgin	8.1					
Federation	7.9	6.1	4.5	6.7	4.9	
Rex	8.1		4.6		4.9	
Comanche	8.0		4.6			3.4
Elgin[d]		5.9	4.5	6.5	5.0	2.5
Baart			4.4			

[a] Acid equivalents of orange G dye/g. protein $\times 10^4$.
[b] Heated 3 to 4 minutes at neutral pH.
[c] Dispersed in normal acetic acid after extraction of fraction A with phosphate buffer solution.
[d] Sample of different year and source.

fraction are within the experimental error ($\pm 0.1 \times 10^{-4}$) except for fraction D. The protein of fraction D is nondispersible in dilute acetic acid and necessarily contains about 98% starch. Since the starch also binds a small amount of dye (4.5 mg. dye/g. of starch) there is a sizable correction factor which may account for some of the differences. Another factor which is consistent with the differences is the amount of protein extracted by the dilute acetic acid. Comanche, Rio, Elgin, and Golden varieties will disperse 84, 87, 91, and 91% of their total

protein, respectively, when the flour is dispersed in dilute acetic acid. These values are constant for a given sample of flour over a wide range of acetic acid concentration, at least between 0.02 *N* and normal. If it is assumed that the lower percentages of total protein dispersed can be attributed to nondispersed B fraction, which has a higher binding capacity, then the differences in the binding capacity for fraction D are eliminated, and a limiting value of 2.4×10^{-4} acid equivalents/g. of protein is obtained.

Fraenkel-Conrat and Cooper gave values of 4.3 and 5.5×10^{-4} acid and base equivalents/g. gliadin protein with orange G and safranin O, respectively. These values compare favorably with fraction B which contains most of the so called gliadin and some less soluble gluten components. Values of the binding capacity of fraction B are of special interest and were a major reason for this investigation. Using purified gluten, some extensive viscosity studies (6) gave evidence that varietal differences in gluten were obtained only when the conditions were favorable for electrostatic forces on the protein molecules to interact. As seen from the constancy of the binding values for fraction B in tables I and II, it does not appear likely that the electrostatic differences referred to can be ascribed to the primary charges associated with the basic and acidic groups of the protein molecules. Although an equal number of reactive sites appears to exist, this does not exclude varietal differences that may result from a nonuniform distribution of these sites on the protein molecule. This does not seem likely, however. Consequently, it is more likely that other electrostatic forces of a weaker nature (incapable of dye-binding) are responsible for the varietal differences of gluten. These differences are not observed when the gluten is dispersed in aqueous 8% sodium salicylate which gives electrostatic equality to gluten dispersions, thereby obliterating differences in the glutens with respect to their viscosity behavior (6).

Although Fraenkel-Conrat and Cooper (1) have shown that denaturing of egg albumin by heating to 70°C. for 5 minutes at pH 2.5 or 11.7 did not affect subsequent dye-binding capacity, a somewhat different means of denaturing the protein of fraction A by heating did result in a consistent lowering of its dye-binding capacity as shown in Table II. The chief difference is that fraction A was heated in a neutral medium at the boiling point for 3 to 4 minutes or until the protein precipitated. Similar heating of fraction B at pH 2.2 did not precipitate the protein, and no effect on its dye-binding capacity was observed. However, when fraction B was dispersed in normal rather than 0.03 *N* acetic acid, after having been washed out with a buffer solution, a consistent increase in its binding capacity resulted. That

this is probably a denaturing effect attributable to the combination of washing with buffer followed by dispersion in moderately concentrated acetic acid is substantiated by the fact that fraction C as obtained by dispersing the flour directly in normal acetic acid is not altered in its capacity to bind dye.

When dispersions of fraction B or C were not neutralized prior to addition of the dye solution, subsequent centrifuging did not yield a clear solution for all varieties. The reasons for this are not understood, since the omission or inclusion of this step does not affect the dye-binding capacity of the protein, provided a clear solution is obtained after centrifuging.

The constant dye-binding capacity of fraction C is somewhat surprising, since this would indicate a fairly constant ratio between fractions A and B. A calculation shows that fraction C is composed of 87% fraction B and 13% fraction A. It is significant and interesting that the protein complex should be composed of nearly proportionate amounts of two fractions—high in basic group content on the one hand and low in basic group content on the other. Further work is anticipated in conjunction with this phenomenon.

Fraction A was further subdivided into a soluble albumin fraction by the method of Pence and Elder (3). The dye-binding capacity of this fraction is 8.8×10^{-4} acid equivalents/g. protein, and is a little higher than that of fraction A. This presumably results from the removal of a small amount of some gluten components which have a lower dye-binding capacity.

Reasoning similar to the above would suggest that differences in baking attributed to the soluble components of flours by Pence *et al.* (4) are not likely to be associated with the primary electrostatic forces of the soluble proteins but with some other component or protein property. It must be remembered, however, that the mild pH conditions used in the baking experiments are much different than the high and low pH used with dye-binding. This may be a significant factor in the interpretation of these results.

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DIFFERENCES IN THE DISTRIBUTION OF COMPONENTS IN ALBUMIN PREPARATIONS FROM DURUM AND COMMON WHEAT FLOURS[1]

JAMES W. PENCE, N. E. WEINSTEIN, AND DALE K. MECHAM

ABSTRACT

The relative amounts of individual components in albumins prepared by a standardized procedure from durum wheat flours differed characteristically from those observed in similar preparations from club and common wheat flours. Visual comparisons of paper electropherograms showed durum preparations to contain large amounts of the alpha group of components and only small amounts of the beta and gamma albumins. In preparations from club and common wheat flours the beta group was present in the greatest amounts, the beta-2 and beta-3 components being characteristically prominent. These differences in component distribution appear to be independent of differences in growth location and crop year.

Varietal differences of a relatively minor nature were observed among preparations from flours of five club and common wheat varieties, although the component distribution patterns for these flours were essentially similar. The beta-1 albumin, a relatively minor component, appeared to be absent from preparations of Chiefkan and Elgin varieties. Preparations from Baart and Hymar flours tended to be low in the alpha-1 and alpha-2 components, and Rex preparations were low in the higher gamma components.

The albumin proteins of wheat flour appear to be a group of components, quite similar with respect to properties such as sedimentation behavior or tryptophan and amide nitrogen content, but significantly different with respect to electrophoretic behavior (2, 3). As many as 11 different components have been detected by electrophoretic examination of preparations isolated from flour extracts by fractional precipitation with ammonium sulfate (2). Visible differences on paper electropherograms showed that the individual components in an albumin preparation were present in greatly different amounts. Also, the relative distribution of components in a preparation from a durum flour was markedly different from that obtained from a common wheat flour (2). Because of the genetic differences between durum and common wheats and because of the pronounced inferiority of durum flours for breadmaking, the observed differences in electrophoretic distribution of components in preparations from the two types of wheat stimulated the more extensive comparison of albumins from other durum and common wheat flours described in the present paper.

Materials and Methods

The flours were selected from the series of experimentally milled,

[1] Manuscript received April 6, 1954. Contribution from Western Utilization Research Branch, Albany, California, Agricultural Research Service, U. S. Department of Agriculture.

TABLE I

DESCRIPTION OF FLOURS USED FOR PREPARATION OF ALBUMINS

Variety	Type	Crop Year	Growth Location	Protein[a]
				%
Turkey	Common, HRW	1946	Texas	13.4
Red Chief	Common, HRW	1946	Texas	12.9
Comanche	Common, HRW	1946	Kansas	9.7
Thatcher	Common, HRS	1946	Montana	14.2
Chiefkan	Common, HRW	1946	Washington	9.6
Chiefkan	Common, HRW	1948	Moro, Oregon	8.4
Chiefkan	Common, HRW	1948	Pendleton, Oregon	8.8
Chiefkan	Common, HRW	1949	Moro, Oregon	9.5
Baart	Common, white	1946	Washington	9.5
Baart	Common, white	1948	Moro, Oregon	8.6
Baart	Common, white	1948	Pendleton, Oregon	8.2
Baart	Common, white	1949	Moro, Oregon	9.8
Rex	Common, white	1946	Washington	7.8
Rex	Common, white	1948	Moro, Oregon	9.5
Rex	Common, white	1948	Pendleton, Oregon	6.9
Rex	Common, white	1949	Moro, Oregon	8.3
Hymar	Club	1946	Washington	5.7
Hymar	Club	1946	Washington	9.0
Elgin	Club	1948	Moro, Oregon	7.2
Elgin	Club	1948	Pendleton, Oregon	6.2
Elgin	Club	1949	Moro, Oregon	7.0
Pentad	Durum	1946	North Dakota	11.6

[a] N × 5.7 at 14% moisture.

unbleached flours described in a preceding paper (4) and are listed in Table I. The flours have a wide range in baking properties and include representatives of the major types of wheat grown commercially. These flours had been held in frozen storage (— 10°F.) for a number of years, but baking tests indicated that they had not deteriorated in baking performance during the storage period.

A series of durum semolinas was used which consisted of six varieties from different growth locations in North Dakota during the 1952 crop year. A second series of semolinas (reduced to flour) from seven varieties of durum wheat and flours from five varieties of hard red spring wheat grown at two locations during the 1953 crop year were also used (Table II). The 1952 semolinas were ground in a laboratory hammer-mill to pass a 40-mesh sieve before use.

Albumins were prepared from flour by methods which have been described previously (3). Water-soluble constituents were extracted with pH 6.8, 0.1% phosphate buffer, and albumins were separated from gliadin and soluble polysaccharides by fractional precipitation between 0.4 and 1.76 *M* ammonium sulfate.

TABLE II

DESCRIPTION OF DURUM SEMOLINAS AND HARD RED SPRING FLOURS USED FOR PREPARATION OF ALBUMINS

Variety	Type	Year Crop	Growth Location in North Dakota
Pentad	Durum	1952	Fargo
Stewart	Durum	1952	Edgeley
Mindum	Durum	1952	Fargo
Nugget	Durum	1952	Minot
Vernum	Durum	1952	Edgeley
L. D. 358	Durum	1952	Minot
Thatcher	HRS	1953	Fargo
C. I. 186	HRS	1953	Fargo
Minn. 2854	HRS	1953	Fargo
Mida	HRS	1953	Fargo
Mida	HRS	1953	Langdon
Stewart	Durum	1953	Langdon
Stewart	Durum	1953	Fargo
L. D. 356	Durum	1953	Fargo
Pentad	Durum	1953	Fargo
Mindum	Durum	1953	Fargo
Vernum	Durum	1953	Fargo
Nugget	Durum	1953	Fargo

Electrophoresis on paper was conducted by the method of Kunkel and Tiselius (1) using Schleicher and Schüll No. 598 or Whatman No. 3 MM filter paper and a pH 6.0 cacodylate buffer (0.02*M*) whose ionic strength was adjusted to 0.20 with sodium chloride. Runs were conducted for 24 hours using approximately 4.6 volts per cm. at 27° to 29°C. Under these conditions, the components in 0.04 ml. of 4% solutions of the albumins were spread into patterns up to about 28 cm. in length.

Results and Discussion

Paper electropherograms for albumin preparations from several types of flour are shown in Fig. 1. In general, the distribution patterns of the various components are similar for most of the flours. The outstanding exception is the pattern for the durum flour, Pentad variety. In this pattern, the relative absence of all components except those of the alpha group, the beta-1, and small amounts of the beta-2 albumins contrast sharply with the large amounts of beta-2 and beta-3 albumins which predominate in the patterns for the other flours. That this pattern is more or less characteristic of durum flours is shown by Fig. 2. Without exception, the durum varieties show the prominence of the alpha group and beta-1 components and the virtual absence of the beta-2 and beta-3 components.

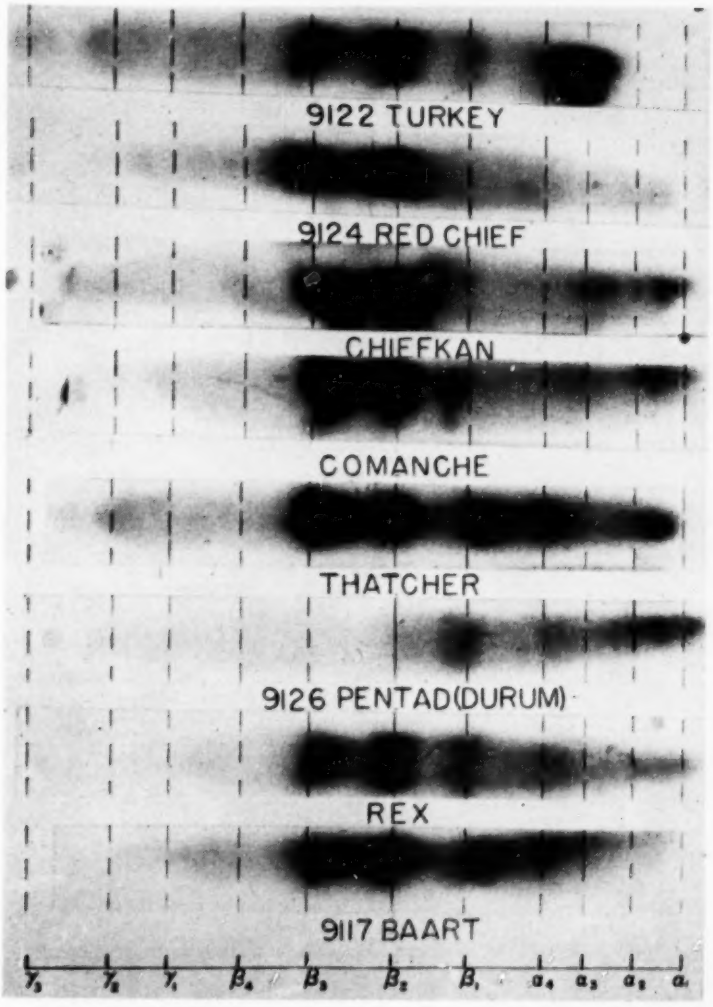

Fig. 1. Paper electropherograms showing relative amounts of individual components in albumin preparations from flours of different wheat varieties.

Various differences of a relatively minor nature can also be seen among the patterns in Figs. 1 and 2. For instance, preparations from the 1952 durum semolinas (last six patterns in Fig. 2) appear to be relatively deficient in the alpha-3 component, as compared to the preparation from No. 9126 Pentad flour (third pattern, Fig. 2). There

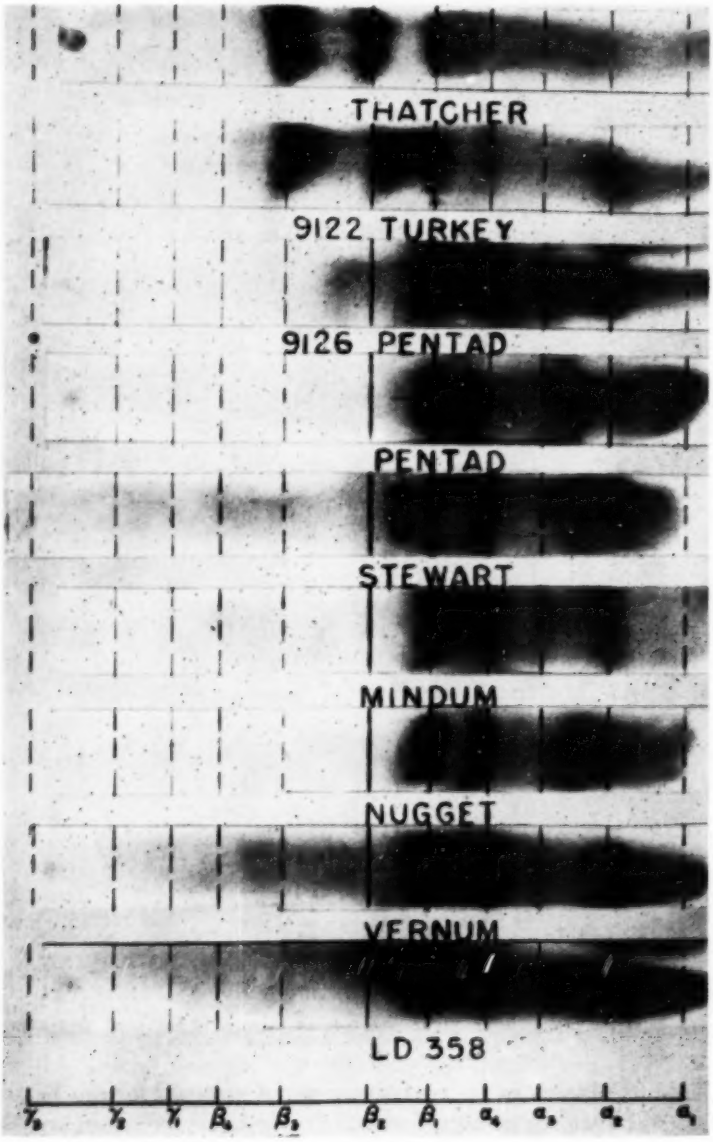

Fig. 2. Paper electropherograms comparing relative amounts of individual components in albumin preparations from flours of common wheat varieties and semolinas of durum wheat varieties. Areas outlined with pencil represent protein denatured at the origin.

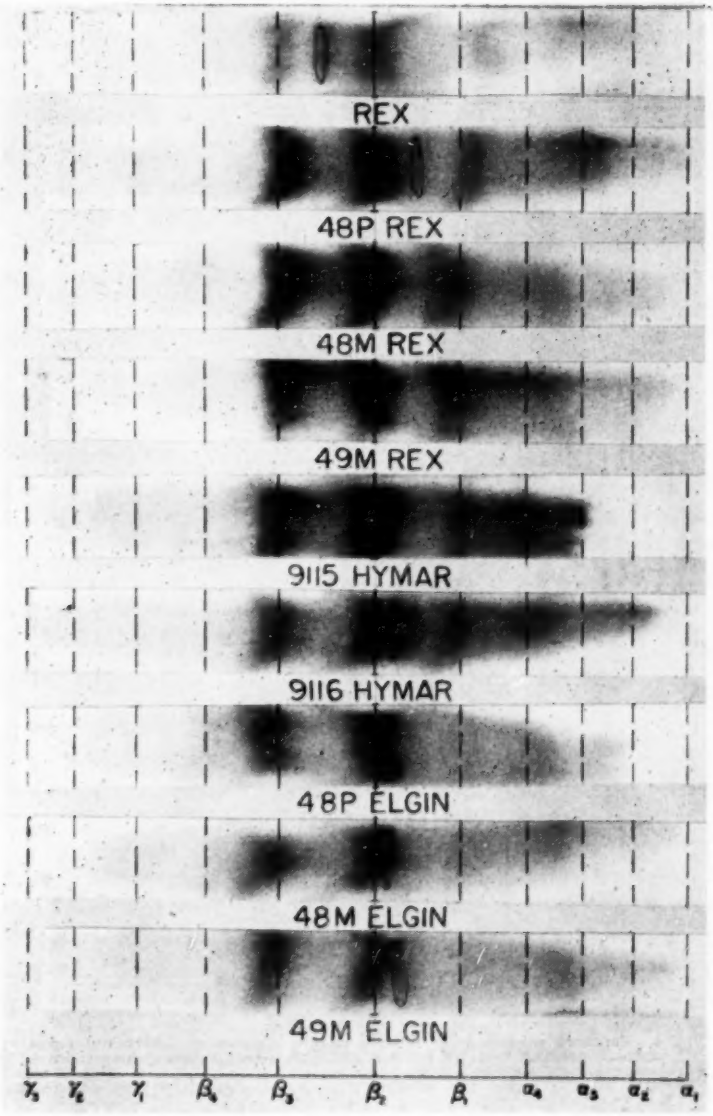

Fig. 3. Paper electropherograms showing relative amounts of individual components in albumin preparations from flours of different common wheat varieties. Areas outlined with pencil represent protein denatured at the origin.

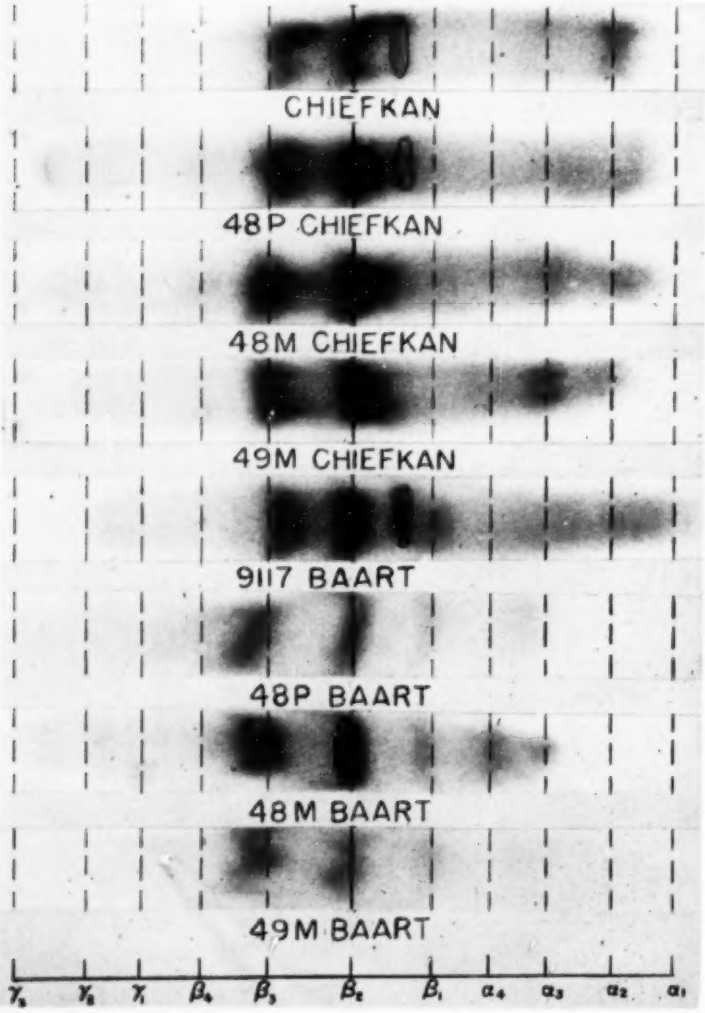

Fig. 4. Paper electropherograms showing relative amounts of individual components in albumin preparations from flours of common and club wheat varieties. Areas outlined with pencil represent protein denatured at the origin.

is also variation in the amounts of beta and gamma albumin among the durum preparations. With the preparations from common wheats, variations seem to concern the various alpha and gamma components, chiefly; but no generalizations of any consequence are apparent.

In Figs. 3 and 4 are shown paper electropherograms of albumin preparations from several varieties for which two to four different flours were available. Again, it is seen that distribution patterns of the components are essentially similar, a fact which further emphasizes the uniqueness of the patterns from durum flours. In addition, a few minor differences can be seen which seem to be confined to certain varieties. In both Chiefkan and Elgin preparations, the virtual absence of beta-1 albumin is noteworthy. The beta-1 component is seldom present in large amounts except in preparations from durum flours, but it is usually a well-defined component, easily located on the electropherograms. Preparations from the Baart and Hymar flours tend to be low in the alpha-1 and alpha-2 albumins, whereas Rex preparations appear deficient in the higher gamma components. No consistent differences are apparent between club and common wheat varieties.

The characteristic differences between albumins from durum and common wheats appear to be unaffected by differences in growth location and crop year. Figure 5 shows patterns for albumin preparations from three varieties of hard red spring wheats and five durum wheats grown at the same location (Fargo, N.D.) during the 1953 crop year (compare also with durum patterns for 1952 crop year in Fig. 2). Figure 6 shows patterns for a hard red spring and a durum variety grown at two different locations (Fargo and Langdon, N.D.) during the 1953 crop year. The major differences between the two types of wheat are consistent among all the samples, although it was noticed that the 1953 durum samples show definitely greater amounts of the alpha-3 component than are shown by most of the 1952 durum samples in Fig. 2.

It is recognized that although certain differences in the albumin composition of flours have been demonstrated, still other differences may exist among the proteins in unfractionated extracts of flours. As discussed previously (3), pentosans and other polysaccharides in a flour extract prevent adequate electrophoretic resolution of the protein components, and removal of the soluble carbohydrates by presently known methods causes a loss of roughly one-fourth of the protein. Nevertheless, a certain amount of information can be obtained by electrophoresis of dialyzed flour extracts from which gliadin, only, has been removed by precipitation with 0.4 *M* ammonium sulfate (3). In Fig. 7 are shown electropherograms of typical extracts and corresponding albumin preparations from two flours. Although resolution with the extracts is poor and the patterns are faint, it can

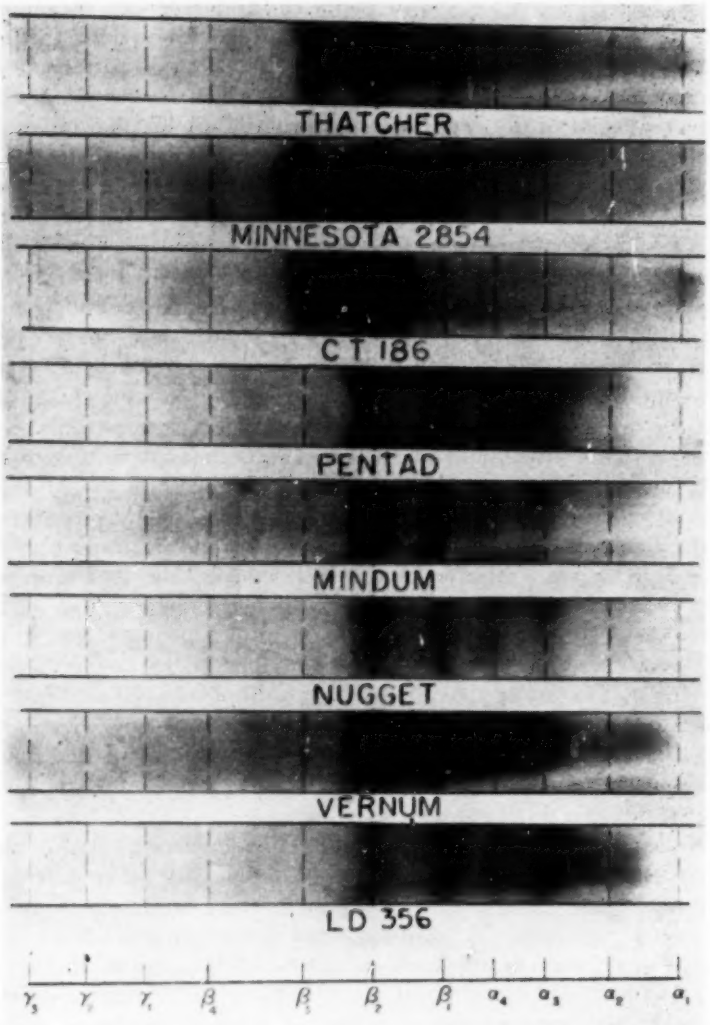

Fig. 5. Paper electropherograms showing relative amounts of individual components in albumin preparations from flours of durum and hard red spring varieties grown at the same location (Fargo, N. D.) during the same crop year (1953). Areas outlined in pencil represent protein denatured at the origin.

be seen that the two types of preparations differ mainly in two aspects. The relative amounts of gamma albumins in the extracts are larger than in the albumin preparations, but the proportions of beta-2 and beta-3 components are smaller, particularly with the club wheat flour.

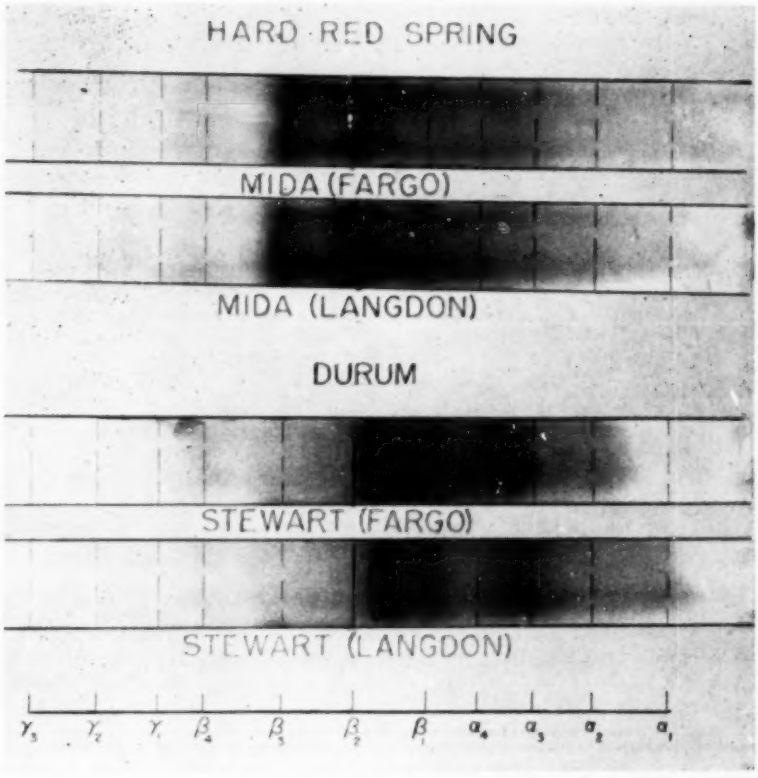

Fig. 6. Paper electropherograms showing relative amounts of individual components in albumin preparations from flours of a durum and a hard red spring variety grown at two locations (Fargo and Langdon, N. D.) in the same crop year (1953). Areas outlined in pencil represent protein denatured at the origin.

However, major differences in the albumin composition of durum and club (or common) wheat flours are still evident. Efforts are being continued to improve the electrophoretic resolution of protein components in flour extracts and to obtain suitable quantitative measurements of the differences in albumin composition of flours.

The foregoing demonstration of consistent differences in the albumin composition of durum and common wheat flours raises the question of whether these differences may be related to the inferior bread-baking properties of durum wheats. A satisfactory answer to this question, however, will require additional information regarding properties of the albumins and their contributions to dough properties.

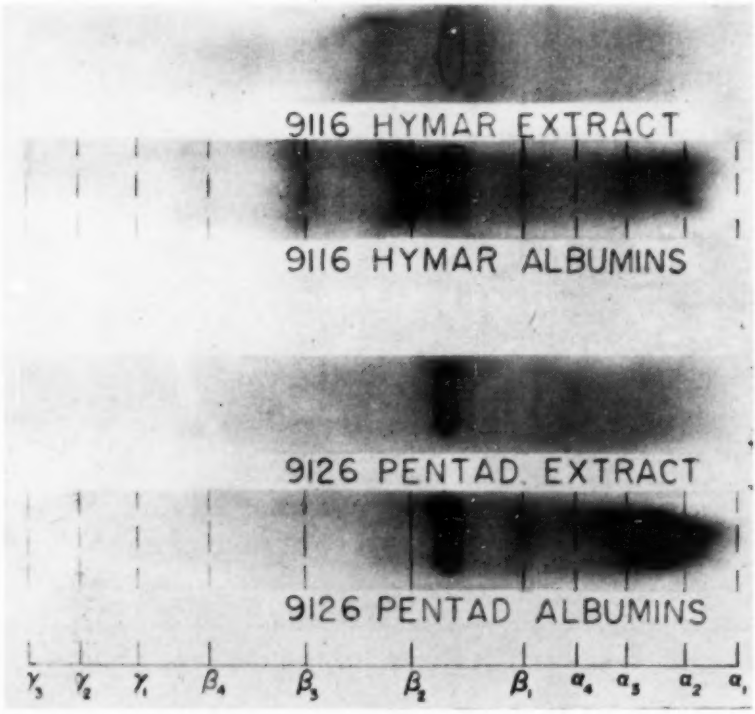

Fig. 7. Paper electropherograms comparing relative amounts and electrophoretic resolution of components in albumin preparations and in unfractionated extracts of wheat flours.

Acknowledgments

We wish to express gratitude to Dr. R. H. Harris of the North Dakota Agricultural Experiment Station, Fargo, North Dakota, for the samples of durum semolina and hard red spring flours used in this study. We are also indebted to Mr. Roy K. Durham and the Millers' National Federation; to Mr. E. J. Bell of the Oregon Wheat Commission; and to the Hard Red Winter Wheat Quality Laboratory, Manhattan, Kansas, and the Western Wheat Quality Laboratory, Pullman, Washington, both of the Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture, for the remainder of the flours used in this work.

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INVESTIGATIONS ON THE QUALITY OF DIPLOID AND TETRAPLOID RYE FOR BREADMAKING[1]

H. M. R. Hintzer and H. de Miranda[2]

ABSTRACT

The baking quality of eight varieties of diploid rye grown at six locations in the Netherlands in 1948 was correlated with maltose value and amylase activity (both negatively), with maximum viscosity in the amylograph (positively), and with other properties associated with sprouting. Large interfield differences were found, whereas varietal differences were small.

Tetraploid rye was higher in protein content, lower in alpha-amylase activity, and superior in baking quality to diploid rye from different fields. The data indicate that tetraploid rye may be the more resistant to sprouting.

In western Europe the main objective in rye breeding is to improve yield, and such factors as disease resistance, winter hardiness, resistance to sprouting, and strength of straw are of direct importance. However, very little attention is given to baking quality of the grain in spite of the fact that in Scandinavia and in many districts of Germany the larger part of the rye crop is used for human consumption. Rye bread is eaten widely in the southern and eastern provinces of the Netherlands and the question of baking quality cannot be ignored.

The proper consideration of quality in a rye breeding program requires an adequate knowledge of the structural features and of the chemical and technological properties of the rye kernel which have an influence on baking quality. Although there is only limited knowledge in this field, important contributions have been made by investigators in western Europe during the past few decades. The extensive experiments of the Norwegian investigator, Schulerud (13), led him to stress the significance of starch and the starch-splitting enzymes; in the manufacture of rye bread, a correct balance between degree of gelatinization and amylolysis is essential for the formation of an elastic, porous, and uniform bread crumb. German investigations (8, 11, 12) with the Brabender Amylograph (2) have led to similar conclusions.

Aust and Ossent (1) ascertained that German varieties with a high protein content tended to exhibit a medium to low maltose-forming capacity, a property which served as a measure of the resistance of the grain to sprouting.

Pelshenke (10) and, later, Hintzer (4) studied several varieties grown in various districts and concluded that the degradation of starch

[1] Received for publication March 22, 1954. Presented at the Annual Meeting, May, 1954.
[2] Cereals Department of the Central Institute for Nutrition Research, T. N. O., Wageningen, The Netherlands.

during baking is predominantly influenced by conditions of growth, although there were indications that alpha-amylase activity also is a genetic factor.

In recent years, there has been considerable interest in tetraploid rye (3, 8, 14), but very limited information on its value for breadmaking is available. Kernels of tetraploid and diploid rye are illustrated in Fig. 1. The studies of Ljung (7) and of Müntzing (8) have shown that the tetraploid strains were higher in protein, ash, and crude fiber than the diploid strains and yielded bread of better volume, texture, and crumb color.

Fig. 1. Photographs of kernels of tetraploid (left) and diploid rye (right) showing the much larger size of the former.

The present studies were undertaken to secure additional information on the effect of variety and environment on the quality of diploid rye for breadmaking and to determine the relative quality of diploid and tetraploid rye grown in the Netherlands.

Materials and Methods

Two series of samples were employed. The first comprised 48 samples of diploid rye representing eight varieties (four Dutch, two German, one Belgian, and one Swedish), each grown in six districts in 1948. The second series consisted of 21 samples of tetraploid rye and nine samples of diploid rye grown in the crop years 1948 to 1951 inclusive. Strictly comparable diploid and tetraploid samples could not be obtained because they must be grown in different fields to prevent cross-pollination.

The investigation of these samples included determinations of hectoliter weight, 1000-kernel weight, protein content, alpha-amylase, maltose figure, amylogram properties, and the conduction of baking tests. With the exception of the first two properties, the investigations were carried out on fairly white flour (ash content, approximately 0.7%, dry matter basis) obtained by Bühler milling.

Hectoliter weight (kg. per hectoliter) and 1000-kernel weight (in g.) were determined in the usual way and the protein content was determined according to a modification of the Kjeldahl procedure, using a conversion factor of 5.83.

The alpha-amylase content was determined according to Hoskam (6). This method involves measurement of the decrease in color intensity with iodine when an aqueous extract of the flour or meal acts upon beta-limit dextrin solution at pH 5.3 and 30°C.

The maltose figure was determined according to the Rumsey procedure as modified by van der Lee (9), which involves the autolysis of the flour suspension for 60 min. at 27°C. The results are expressed as g. of maltose per 100 g. flour or meal. The corrected maltose figure is obtained by subtracting the reducing sugars (as maltose) originally present in the sample.

The amylogram, made by the Brabender Amylograph, represents the course of the viscosity of a suspension of 60 g. flour in 450 g. water upon heating to about 95°C., the temperature being raised at a rate of 1.5°C. per minute. From the amylograms, the following data were derived: 1) the maximum viscosity, expressed in Brabender units (B.U.); 2) the temperature (°C.) at which maximum viscosity is reached; and 3) the percentage decrease of the viscosity 5 minutes after the maximum.

Baking tests were carried out with rye flour and with rye meal. With the flour, a white pan bread was baked, which is normally not made in the Netherlands, but which in Denmark, Norway, and various districts in Germany is a popular food. This type of baking test is very attractive, since differences in quality are more clearly revealed by the better-developed shape and texture than in the case of brown rye bread, thus making a more precise evaluation possible. In the southern provinces of the Netherlands, rye meal is finely milled; in the eastern provinces, a more coarsely milled rye meal is made. Both are used for the preparation of a brown rye bread. In our experiments, the finely milled meal was baked to a brown hearth bread.

The formula for the baking test with rye flour was as follows: 100 parts (by weight) of flour, 2 parts of yeast, 2 parts of salt, and 61 parts

of water were mixed for 5 minutes, followed by a first fermentation of 15 minutes (dough temperature 26° to 27°C.). The dough was divided into pieces for loaves of 600 g. and molded for pan bread. After a proof of 45 minutes, the loaves were baked for about 45 minutes at about 220°C.

In the baking test with rye meal, the following formula and procedure were used: 100 parts (by weight) of meal, 1 part of yeast, 1 part of salt, and 48 parts of water were mixed for 10 minutes, followed by a fermentation of 50 minutes (dough temperature 26° to 27°C.). The dough was divided into pieces for loaves of 650 g. and molded for oven plate loaves. After a proof of 45 minutes, the loaves were baked for 120 minutes at a temperature decreasing from about 210° to about 150°C.

The loaves were judged one day after being baked. Attention was paid to volume, shapeliness, color, and bloom of the crust and color and texture of the crumb. Great differences were especially noticeable in the degree of stickiness of the crumb. This quality was judged in two ways: viz., by external examination and by measurements. The external examination was expressed as a score number (higher according to the better quality of the bread, thus less sticky). The measurements were carried out with a compressimeter (panimeter) (5). A cylinder cut out of the bread is compressed by this instrument with gradually increasing stress; then the pressure is released and observation is made as to what percentage of the original height the bread cylinder recovers. This percentage, called "panimeter value," is a measure of crumb elasticity or lack of stickiness.

The percentage of soluble substance in the bread crumb was determined by extracting a certain amount of finely crumbled bread with water. After filtration the extract was evaporated on a water bath and dried to constant weight at 105°C. The results are expressed on a dry matter basis.

Results

Series I. Diploid Varieties, 1948 Crop. The mean analytical data for all diploid varieties grown at each of the six stations are recorded in Table I; the corresponding mean values for the eight individual varieties over all stations are given in Table II.

The station means show that the conditions under which the rye was grown had a great influence on baking quality and the various measures of amylase activity. These differences are to be attributed to the degree of sprouting, which has an important bearing on the level

TABLE I

ANALYTICAL DATA FOR DIPLOID RYE SAMPLES, 1948 HARVEST
FIELD MEANS FOR SIX VARIETIES

Properties	Experiment Field						
	E	C	A	B	D	F	Range[a]
Protein content (d. m. basis), %	7.9	7.0	7.7	6.9	8.3	8.2	1.6
Alpha-amylase activity, Hoskam units	0.85	1.10	1.70	2.40	3.81	9.34	8.49
Maltose figure (corrected), g maltose/100 g flour	0.75	0.89	0.99	0.92	1.38	1.24	0.63
Maximum viscosity, B.U.	673.00	647.00	528.00	506.00	414.00	365.00	308.00
Temperature at maximum viscosity, °C.	65.3	63.4	62.0	60.7	59.7	58.4	6.9
Viscosity decrease from maximum, %	34.5	36.1	49.3	60.0	57.0	67.9	33.4
Panimeter value (white bread)	78.0	75.0	74.0	67.0	62.0	53.0	25.0
Soluble substance (white bread, d. m. basis), %	23.6	23.8	27.8	31.0	33.4	41.8	18.2
Stickiness score (white bread)	3.63	3.31	3.06	1.88	1.31	0.50	3.13
Stickiness score (brown bread)	2.25	2.25	2.13	1.88	1.63	0.50	1.75

[a] Difference between maximum and minimum values.

of alpha-amylase activity. The samples from field F, which had the highest amylase activity, showed sprouts on visual examination; although sprouts were not clearly detected in the samples from the other fields, the analytical data show rather marked differences.

The varietal means over all stations show only minor differences; the only properties which differed significantly were the maltose figure and the degree of elasticity of white bread as determined with the panimeter.

Simple correlations of alpha-amylase activity and of panimeter value with the various properties were obtained for varieties, stations, and the interaction (stations $\times$ varieties) by means of covariance analyses. These coefficients, recorded in Table III, show that those properties which depend upon the action of alpha-amylase on the starch are highly correlated.

Series II. Diploid and Tetraploid Ryes. The most important results of the comparative studies on the samples of diploid and tetraploid ryes are summarized in Fig. 2. With the exception of weight per 1000 kernels and protein content, the various analytical values are, in general, lower for the tetraploid than for the diploid samples. With tetraploid rye, the maximum viscosity, obtained by the amylograph test, is somewhat lower than would be expected from the maltose figure and the viscosity decrease from the maximum; this discrepancy

TABLE II

ANALYTICAL DATA FOR DIPLOID RYE SAMPLES, 1948 HARVEST
VARIETAL MEANS FOR EIGHT FIELDS

Properties	Varieties								
	I	II	III	IV	V	VI	VII	VIII	Range[a]
Protein content (d.m. basis), %	8.3	7.4	7.8	7.8	7.8	7.4	7.6	7.5	0.9
Alpha-amylase activity, Hoskam units	3.10	1.87	2.40	1.74	2.52	2.09	2.35	2.35	1.36
Maltose figure (corrected), g maltose/100 g flour	1.12	0.79	1.07	0.96	1.11	1.12	1.03	1.04	0.33
Maximum viscosity, B.U.	453.00	562.00	566.00	502.00	459.00	573.00	533.00	531.00	120.00
Temperature at maximum viscosity, °C.	59.8	62.8	60.6	61.8	61.0	62.6	61.9	62.1	3.0
Viscosity decrease from maximum, %	60.8	50.2	42.2	48.5	54.2	52.5	48.3	49.7	8.6
Panimeter value (white bread)	63.0	73.0	72.0	73.0	64.0	70.0	67.0	71.0	10.0
Soluble substance (white bread, d.m. basis), %	33.8	27.6	30.7	29.0	32.7	30.8	29.5	27.9	6.2
Stickiness score (white bread)	2.00	2.92	2.33	2.33	2.17	2.00	2.08	2.42	0.92
Stickiness score (brown bread)	1.50	2.00	1.83	1.50	2.00	1.83	1.67	1.83	0.50

[a] Difference between maximum and minimum values.

TABLE III

SIMPLE CORRELATION COEFFICIENTS BETWEEN SELECTED VARIABLES FOR FIELDS, VARIETIES, AND INTERACTION

Property	Correlations with Alpha-Amylase[a]			Correlations with White Bread Panimeter Value[a]		
	Between Fields	Between Varieties	Interaction	Between Fields	Between Varieties	Interaction
Protein content (d. m. basis), %	+0.47	+0.68	+0.26	−0.43	−0.62	−0.24
Alpha-amylase activity, Hoskam units	....	....	....	−0.98**	−0.83**	−0.59**
Maltose figure (corrected), g maltose/10 g flour	+0.83*	+0.68	+0.70**	−0.84**	−0.62	−0.41*
Maximum viscosity, B.U.	−0.97**	−0.53	+0.73**	+0.95**	+0.72*	+0.50**
Temperature at maximum viscosity, °C.	−0.96**	−0.80*	−0.63**	+0.96**	+0.62	+0.43**
Viscosity decrease from maximum, %	+0.94**	+0.51	+0.60**	−0.93**	−0.71*	−0.45**
Panimeter value (white bread)	−0.98**	−0.83*	−0.59**	....	....	....
Soluble substance (white bread, d. m. basis), %	+0.99**	+0.76	+0.83**	−0.97**	−0.84**	−0.50**
Stickiness score (white bread)	−0.98**	−0.56	−0.68**	+1.00**	+0.63	+0.61**
Stickiness score (brown bread)	−0.95**	−0.15	−0.36*	+0.92**	+0.07	+0.40*

[a] In fact, the logarithms of these properties were involved in the calculations, because they showed more rectilinear correlations with the other properties.
** Highly significant; value exceeds 1% point.
* Significant; value exceeds 5% point.

may be associated with the higher protein content and correspondingly lower starch content of the tetraploid rye.

Baking tests were also conducted on this series of samples and the breadmaking value of the tetraploid samples was superior to that of the diploid varieties. This finding is in line with the lower alpha-amylase activity of the tetraploid varieties. The photographs in Fig. 3 show that tetraploid rye gives very good bread, and show also the marked effect of extensive sprouting on the breadmaking value of diploid rye. In this loaf from the diploid sample, it would appear that a normal crumb structure was present originally, but collapsed after the crust formed as a result of the marked acceleration in alpha-amylase activity when the temperature increased during baking.

Discussion

The baking quality of rye in the humid climate of the Netherlands depends primarily on the extent of sprouting which results in an increase in alpha-amylase activity. This activity manifests itself in many ways and can be measured by direct as well as indirect methods with sufficient accuracy to provide satisfactory indexes of the relative breadmaking value of the various samples. This is established by the

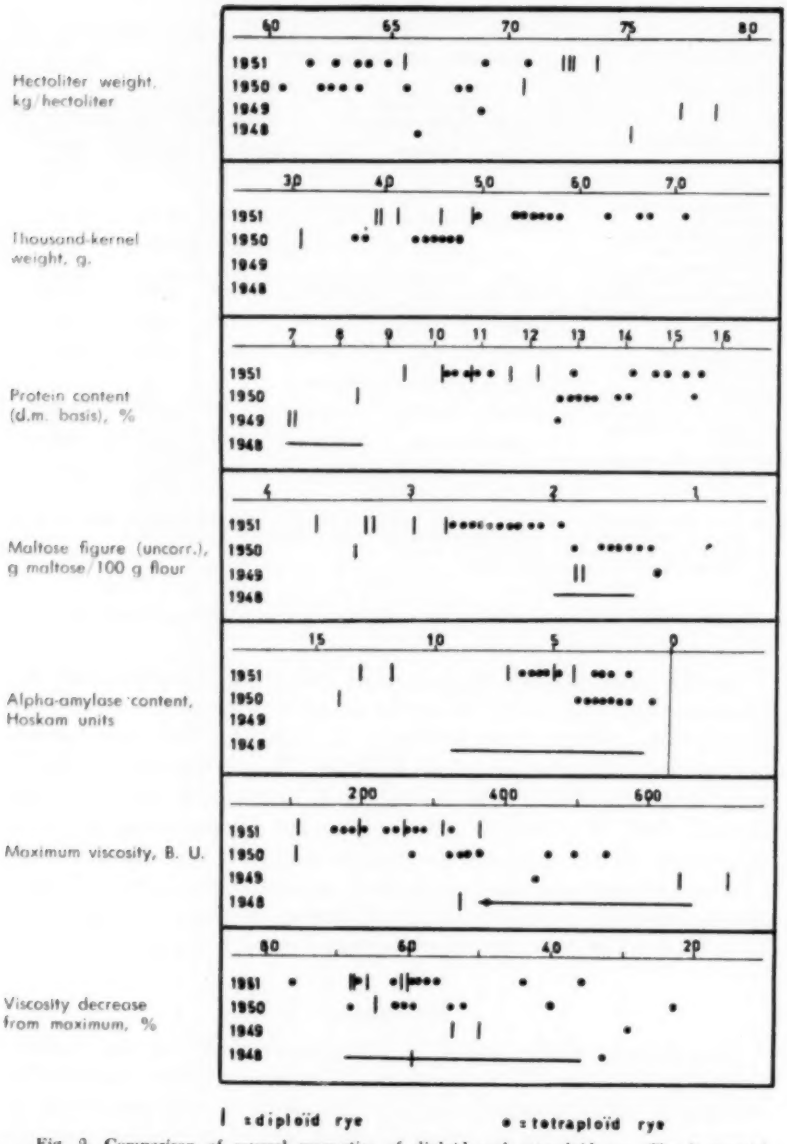

Fig. 2. Comparison of several properties of diploid and tetraploid rye. The horizontal lines indicate the regions of the values of Table I. The units for each property are arranged from left to right in order of their beneficial effects in relation to breadmaking.

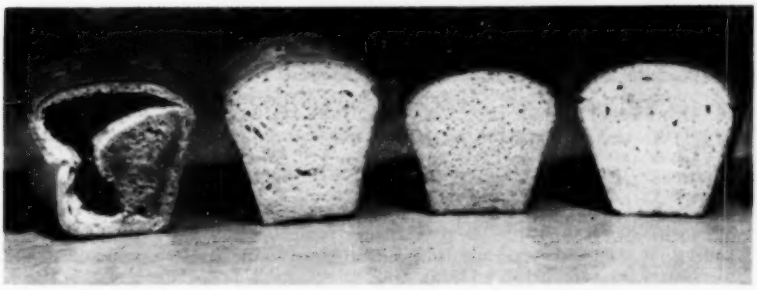

Fig. 3. Loaves of white bread made from rye flour from the 1950 crop. The loaf on the left, which shows extensive starch degradation, was made from diploid rye; the others are from tetraploid rye.

high correlation coefficients which were obtained between these measures and bread quality.

In comparison with environment, variety had relatively little influence on rye quality. This is to be expected because of the great importance of alpha-amylase activity which is increased by humid conditions. While no distinct varietal differences in alpha-amylase activity occurred, some variations in maltose figure and stickiness were noted; this may, perhaps, be due to differences in starch-susceptibility.

In the investigation of the tetraploid rye from various harvests in comparison with diploid rye, the most significant observation was that none of the samples of tetraploid rye showed high amylase activity, whereas several of the diploid samples did so. Although the number of samples was limited and those representing diploid and tetraploid rye did not come from the same field, these observations suggest that tetraploid rye is the more resistant to sprouting. This may be associated with the higher protein content, the larger size of the kernel and consequent increased endosperm content, or to a greater straw strength of the vigorous tetraploid plant, which permits less lodging. Further investigations will be necessary to determine the basic reasons for the differences between the tetraploid and diploid varieties.

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IDENTIFICATION OF THE REDUCING SUGARS IN AMYLOLYSATES OF STARCH AND STARCH-OLIGOSACCHARIDES[1]

J. H. PAZUR AND R. M. SANDSTEDT

ABSTRACT

The reducing sugars produced from starch and starch-oligosaccharides by malt enzymes have been identified by paper chromatographic methods. Alpha-amylase action on starch results in the production of a series of saccharides of low molecular weight, including glucose, maltose, amylotriose, amylotetraose, and amylopentaose; beta-amylase action results in the liberation of maltose as the only product of low molecular weight. Pure amylotriose is hydrolyzed slowly to glucose and maltose by alpha-amylase but is not hydrolyzed by beta-amylase. Pure amylotetraose is hydrolyzed to glucose, maltose, and amylotriose by alpha-amylase and to maltose only by beta-amylase. These action patterns for alpha- and beta-amylase account for the production of glucose, maltose, and amylotriose in digests of starch with malt.

Amylolytic activity in bread-dough results in the formation of reducing sugars of low molecular weight and in the modification of the physical and chemical properties of the residual starch. The reducing sugars are essential for production of carbon dioxide during fermentation, while the modified starch enhances the texture and quality of the baked product. The chemical nature of the sugars and the mechanism of their production by the amylases have not been fully eluci-

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dated (7). In the studies being reported, the reducing sugars in enzymolysates of starch with malt amylases have been identified by paper chromatographic procedures. Several starch-oligosaccharides, amylotriose, amylotetraose, and isomaltose, have been prepared in pure form and have been used to study the mode of action of the malt enzymes.

Materials

Preparation of the Enzymes. Three types of enzyme preparations, (a) wheat malt, (b) wheat malt alpha-amylase, and (c) wheat malt beta-amylase, were employed. The wheat malt sample contained 0.05 unit of alpha-amylase (5) and 0.006 unit of beta-amylase (4) per 1 mg. of material. Alpha-amylase was prepared from the malt by a method utilizing the difference in stability of alpha- and beta-amylase at elevated temperature (8) and in the presence of calcium ions as described by Kneen *et al.* (5). Final purification involved precipitation of the alpha-amylase by the addition of two volumes of acetone (0°C.) and isolation of the precipitate by centrifugation methods. An assay of the dried material showed that it contained 0.5 unit of alpha-amylase per 1 mg. of sample.

Wheat beta-amylase was prepared following the procedure of Kneen and Sandstedt (4). The alpha-amylase in a malt extract was inactivated by maintaining the solution at pH 3.0. The pH of the solution was then raised to 4.8 and the beta-amylase precipitated with two volumes of acetone (0°C.). The dried material assayed 0.15 unit of beta-amylase per 1-mg. sample.

Standard solutions of alpha- and beta-amylase were prepared for studying the action of the enzymes on starch-oligosaccharides. The alpha-amylase solution contained 5 units per ml. while the beta-amylase solution contained 0.15 unit per ml.

Maltose. Since reagent grade maltose contains some glucose and reducing compounds of higher molecular weight as impurities (10), pure maltose was isolated from a digestion mixture of starch and beta-amylase. The limit dextrins in the enzymolysate were removed by precipitation with an equal volume of methanol. The filtrate was concentrated under a vacuum and mixed with Darco G-60 carbon. The maltose was eluted from the carbon with 10% ethanol solution, concentrated by vacuum distillation, and stirred with 5 volumes of hot ethanol. Crystalline maltose was obtained from this solution. Paper chromatographic examination of the maltose showed that the preparation was free of other reducing sugars.

Amylotriose. The isolation of amylotriose, a trisaccharide of three glucose units joined by alpha-1,4-glucosidic bonds, from an enzymolysate of amylodextrin and salivary amylase, has been described in a recent publication (9).

Amylotetraose. The amylotetraose, a tetrasaccharide of four glucose units joined by alpha-1,4-glucosidic bonds, was isolated from a digest of salivary amylase and amylodextrin as described in a previous publication (9).

Isomaltose. Isomaltose, a disaccharide of two glucose units joined by an alpha-1,6-glucosidic bond,[2] was isolated from a partial acid hydrolysate of dextran. The hydrolysate was prepared by refluxing 2 g. of dextran[3] in 15 ml. of 0.07 *N* hydrochloric acid for 6 hours. The solution was neutralized with solid sodium carbonate and concentrated to a volume of 5 ml. The compounds in this concentrate were separated on paper chromatograms (11). The yield of isomaltose was 0.25 g. The specific rotation of this material was found to be $+116°$ (*c* 2, water): literature $+120°$ (6). On paper chromatography, the preparation gave one distinct spot with an R_f value identical with that of pure isomaltose (6, 10). Glucose was the only hydrolytic product detectable in partial and complete acid hydrolysates of the compound.

Starch. In all of the experiments, Merck soluble starch dispersed in boiling water and cooled to room temperature was used as the substrate for the enzymes.

Methods

Paper Chromatography. The paper chromatographic method described by French *et al.* (2) was used for identification of the reducing sugars. A 0.01-ml. sample was placed on the paper chromatogram and developed in *n*-butanol-pyridine-water mixture (6:4:3 by volume) by the multiple ascent technic. For the resolution shown in Figs. 1, 2, and 3, three ascents of the solvent were required. The finished chromatograms were sprayed with alkaline copper reagent (13), heated at 100°C. for 10 minutes, and sprayed with phosphomolybdic acid reagent (14). The areas at which reducing sugars were present appear as blue spots on the paper. For identification purposes, a reference mixture of oligosaccharides containing glucose, maltose, amylotriose, amylotetraose, and higher saccharides of the amylose series was chromatographed routinely with the samples of the digests.

Starch Digests. Six milligrams of malt alpha-amylase (3 units) were

[2] The name "brachiose" has been suggested for this compound by Montgomery et al. (6).
[3] The sample of dextran was kindly supplied by Dr. Allene Jeanes, Northern Regional Research Laboratory, Peoria, Illinois.

added to 100 ml. of starch solution (4%). The mixture was shaken, held at 30°C. for 4 hours,[4] and then heated at 100°C. for 10 minutes in order to arrest enzyme action. The reducing sugars present in the digestion mixture at 0 and 4 hours were separated on paper. A photograph of the finished paper chromatogram is shown in Fig. 1.

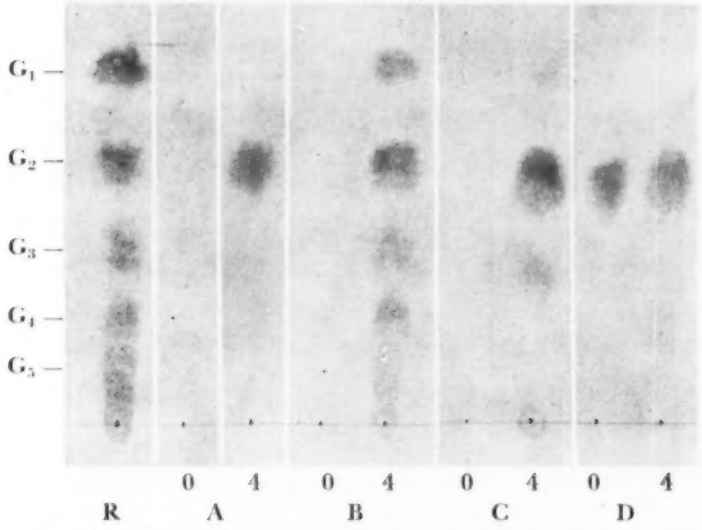

Fig. 1. Reducing sugars in digests of starch with beta-amylase (A), starch with alpha-amylase (B), starch with malt (C), and maltose with malt (D), at 0 and 4 hours; G_1, glucose; G_2, maltose; G_3, amylotriose; G_4, amylotetraose; G_5, amylopentaose; and R, reference oligosaccharides.

Two additional experiments were performed as described above using 2.4 mg. of wheat malt beta-amylase (0.36 unit) and 60 mg. of wheat malt (3 units of alpha-amylase and 0.36 unit of beta-amylase) as the enzymes. The enzyme and substrate concentrations in these experiments corresponded to that in a flour with 1% malt; i.e., the ratio of malt to flour is 1:100. Photographs of the beta-amylase and malt digests also are shown in Fig. 1.

Amylotriose Digests. Digests of 0.2 ml. of amylotriose solution (4%) and 0.2 ml. of standard solutions of alpha- and of beta-amylase were prepared. Enzyme action in 0.1-ml. aliquots of the digests was stopped after 0 and 4 hours by heating the samples at 100°C. for 5 minutes. The reducing sugars in the aliquots were identified on paper chromatograms (Figs. 2 and 3).

[4] An average value for mixing, fermentation, and proofing periods in which the amylases act upon the starch of the flour.

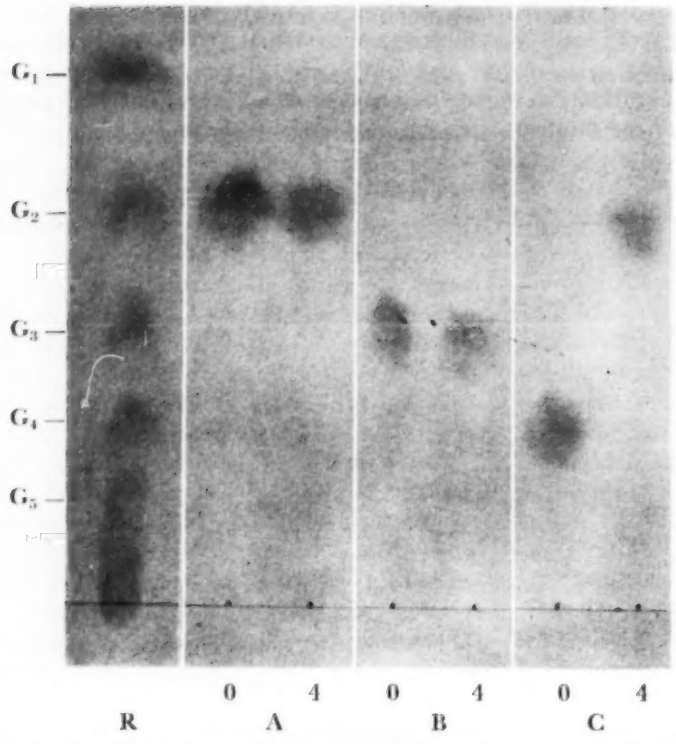

Fig. 2. Reducing sugars in digests of maltose (A), amylotriose (B), and amylotetraose (C), with beta-amylase at 0 and 4 hours. G_1, glucose; G_2, maltose; G_3, amylotriose; G_4, amylotetraose; G_5, amylopentaose; and R, reference oligosaccharides.

Amylotetraose Digests. These digests were prepared following the methods of the previous section. The results of chromatographic analyses at 0 and 4 hours are shown in Figs. 2 and 3.

Maltose Digests. The alpha- and beta-amylase digests were prepared at concentrations of enzyme and substrate used in the two preceding experiments. A malt digest containing 6 mg. of malt in 1 ml. of maltose solution (4%) also was prepared. Samples were analyzed for reducing sugars at 0 and 4 hours. The results are shown in Figs. 1, 2, and 3.

Isomaltose Digests. Digests of isomaltose with alpha-amylase, beta-amylase, and wheat malt were prepared as described for the maltose series. Analyses of these digests showed that the isomaltose was not hydrolyzed by the enzyme preparations.

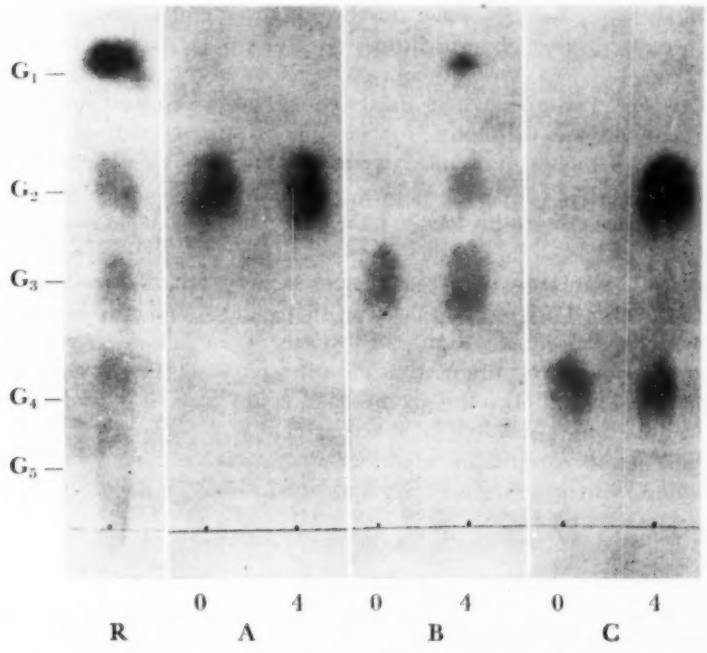

Fig. 3. Reducing sugars in digests of maltose (A), amylotriose (B), and amylotetraose (C), with alpha-amylase at 0 and 4 hours. G_1, glucose; G_2, maltose; G_3, amylotriose; G_4, amylotetraose; G_5, amylopentaose; and R, reference oligosaccharides.

Results and Discussion

Preliminary experiments showed that malt enzymes produced glucose, maltose, and amylotriose from starch. In order to determine the mode of production of these sugars, the action of malt, malt alpha-amylase, and malt beta-amylase was tested on starch and pure starch-oligosaccharides. The results are presented in Figs. 1, 2, and 3.

It is noted in Fig. 1B that a series of sugars of low molecular weight including glucose, maltose, amylotriose, amylotetraose, and amylopentaose was produced from starch by malt alpha-amylase. Since neither maltose nor isomaltose was hydrolyzed by the enzyme preparation, glucose production could not be attributed to the presence of maltase or dextrinase activities in the malt (3). In contrast to the action pattern of malt alpha-amylase on starch, salivary amylase produces only three reducing sugars of low molecular weight, maltose, amylotriose, and amylotetraose (1, 12). Apparently all saccharogenic amylases do not operate by the same reaction mechanism.

Malt beta-amylase converted starch to maltose and a limit dextrin of high molecular weight which does not move on a paper chromatogram (Fig. 1A). Analyses of beta-amylase digests of starch-oligosaccharides (Fig. 2) showed that the enzyme hydrolyzed amylotetraose to maltose but was without action on amylotriose or maltose. Oligosaccharides of higher molecular weight produced by alpha-amylolysis of starch would be converted by beta-amylase to maltose or to maltose and amylotriose depending on whether the oligosaccharide was composed of an even or odd number of glucose residues.

Malt alpha-amylase liberated glucose from starch-oligosaccharides as well as from starch. Figure 3 shows that glucose and maltose were the hydrolytic products from amylotriose, while glucose, maltose, and amylotriose were produced from amylotetraose. The persistence of amylotriose in the two digests indicates a slow rate of hydrolysis of the terminal bonds of starch-oligosaccharides.

These action patterns for alpha- and beta-amylase account for the accumulation of glucose, maltose, and amylotriose in digestion mixtures of starch and malt.

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A METHOD FOR EVALUATING THE WET MILLABILITY OF STEEPED CORN AND GRAIN SORGHUM[1]

STANLEY A. WATSON AND YOSHIRO HIRATA

ABSTRACT

The millability of steeped corn and grain sorghum may be evaluated by the ease of starch removal from the endosperm of thin longitudinal slices of steeped kernels. Sections of 40μ thickness are prepared on the freezing microtome, manipulated in water to remove starch loosened during steeping, and then stained with iodine. The kernel is assessed for relative percentage of horny and floury endosperm and the section is judged for the percentage of starch removed from each of these areas. Millability score is calculated from these data. Applicability of the method to steeping problems is illustrated.

In laboratory studies having to do with steeping and milling characteristics of corn and grain sorghum, evaluation of the milling properties of steeped grain samples must be related to quantitative recovery of starch, the primary product. Actual separation of starch by milling (5) is necessary for final evaluation but the methods are time consuming and lack sensitivity. Cox, MacMasters, and Hilbert (1) showed that examination of thin sections of steeped kernels gave clues to their milling properties. Their method was employed in this laboratory and subsequently simplified and made quantitative so as to provide a numerical score of a sample of steeped grain with regard to pertinent wet milling properties (5). Extensive application has suggested improvements which have now been incorporated into the method to provide a means of evaluating more precisely the potential millability of a steeped grain sample.

Procedure

Apparatus:

Spencer table top microtome with freezing stage
Microtome knife with handle
Camel's-hair brush
Dropping pipette drawn out to a fine tip
Microscope slides
Microscope cover glasses

Reagents:

Gum arabic solution, 10%
Iodine-potassium iodide solution, 0.005 *N* iodine and

[1] Manuscript received April 5, 1954. A contribution from the George M. Moffett Research Laboratories, Corn Products Refining Co., Argo, Illinois.

0.005 *M* potassium iodide
Glycerine
Carbon dioxide (cylinder)

Preparation of Sections. Select 10-20 sound kernels from the steeped grain sample. Examine and trim each kernel with a sharp razor blade by cutting a thin slice off each of the narrow sides. Thus two opposite flat surfaces, the entire length of the kernel, will be exposed. Place a trimmed kernel in a drop of gum arabic solution on the freezing stage and admit carbon dioxide to the freezing chamber. As freezing progresses, place more gum arabic around the kernel so that it is finally encased in the frozen medium. When the kernel is frozen hard, trim the upper surface with the microtome knife until intact sections are reached near the center of the kernel. Then make successive 40μ longitudinal slices until satisfactory sections are obtained. The best procedure is to place a drop of water on the upper knife edge and slowly push the blade over the kernel, so that the section thaws as it is cut. The section will be retained in the drop of water and can be easily picked up with a wet camel's-hair brush, without distortion, for transfer to a shallow dish of water. In the case of very poorly steeped grain, it may be impossible to obtain satisfactory sections. The microtome knife must be maintained at optimum sharpness. A Berkeley Microtome Knife Sharpener, Model C, has been found to be almost indispensable for this purpose.

Select the most intact section and brush under water with a minimum of tearing of the cell wall network to remove all readily releasable starch. This is best accomplished by stroking the section with the side of the camel's-hair brush about 25 times. Transfer the brushed section to a drop of water on a microscope slide. Remove excess water with a dropping pipette pulled out to a fine tip. Then cover the section with several drops of iodine-potassium iodide solution, remove immediately and replace with another drop of the iodine solution. Allow to stain for 30 to 60 seconds. All areas containing starch will become blue or purple. Remove excess iodine solution with a pipette, put a drop of glycerine on the section and a cover glass over it. Allow the remainder of the kernel on the freezing stage to thaw, identify with its 40μ section, and allow it to dry.

Evaluation of Millability Score. The millability score is based upon the completeness of starch removal from the horny and floury endosperm areas. Estimate the percentage of horny endosperm by visually examining the dried remainder of the kernel from which the 40μ

section was cut. For this purpose, horny endosperm is defined as only that part of the kernel which is dense, translucent, or horn-like in appearance. The boundary line between the two areas is chosen at approximately the middle of the transitional zone of any semi-horny area which may lie between the true horny and true floury areas. The proportion of horny area in the endosperm may be estimated visually. The area of the floury endosperm is found by difference. Natural voids or holes in the endosperm (Fig. 1) are not counted as endosperm area. With experience, an observer can estimate the relative areas within 10% of the true value. However, most persons may prefer to obtain a more accurate estimate of areas, at least until some experience has been gained. The authors prefer to do this by projecting an image of the kernel surface onto a ground glass plate with the aid of a short focal length photographic lens. Outlines of the floury and horny areas can then be traced on transparent paper. Their ratios may then be determined from planimeter readings or by cutting out the tracings to obtain ratio of the weights of paper occupied by the horny (or floury) endosperm and entire endosperm areas, respectively.

The next step is to estimate visually the amount of starch released by the brushing operation from the floury and horny areas, respectively. Examine the iodine-stained 40μ section and compare it with the dried kernel surface. Starch removal is expressed as percentage of the area being examined. All blue-stained areas contain starch; all clear areas indicate release of starch. The cut surface area of the dried kernel will generally be smaller than the section because of shrinkage on drying but this should not interfere with the scoring.

To compute the millability score substitute the values which were obtained in the following formula:

$$\text{Millability Score (MS)} = \frac{(2H \times RH) + (F \times RF)}{(2H + F)\,100}$$

Where F = percent of endosperm area that is floury.
H = percent of endosperm area that is horny.
RF = percent of the floury endosperm area from which starch has been released.
RH = percent of the horny endosperm area from which starch has been released.

Wet milling experience and numerous experiments have shown that complete starch removal from the horny endosperm is considerably more difficult to accomplish than it is from the floury endosperm. For example, a kernel having 30% horny endosperm with 100% RF and 0% RH cannot be considered as 70% steeped because more than 30% of the total work required for complete starch removal has yet

to be done. In order to compensate for this difference the horny endosperm has been arbitrarily considered as being twice as difficult to steep as the floury endosperm; therefore, a factor of 2 has been applied to the horny endosperm in the above formula.

Determination of Sample Size. The number of kernels required to obtain valid mean scores cannot be fixed in advance because standard deviation is in general inversely related to millability score. The following procedure has been found useful as a means of determining the number of kernels to score. First determine the mean and standard deviation for the percentage of horny endosperm of the corn sample. To do this, longitudinally bisect with a razor blade 25 or more raw or steeped kernels, discard one-half of each kernel and air dry the other half. Polish the cut surface on a fine whetstone, if necessary, and estimate the percentage of horny endosperm. Next, randomly select 15 steeped kernels and section. When the remainder of the kernel is dry determine the percentage of horny endosperm for each kernel and discard all kernels and their accompanying sections that fall outside the 5% confidence limits (3) determined from the large sample. In most cases 8 to 12 kernels will fall within the confidence limits. All of these kernels should then be evaluated for millability score. Determine the range of scores, i.e., the difference between the highest and the lowest scores, and estimate the standard deviation by dividing the range by the appropriate factor from the following table (4):

No. of Kernels	*Factor for Estimate of S.D.*	*No. of Kernels*	*Factor for Estimate of S.D.*
5	2.33	17	3.59
6	2.53	20	3.73
7	2.70	25	3.93
8	2.85	30	4.09
9	2.97	35	4.21
10	3.08	40	4.32
11	3.17	45	4.42
13	3.34	50	4.50
15	3.47		

If the estimated standard deviation of the millability score is about $\pm$ 10, no more kernels need be sectioned. If it is above $\pm$ 10 then 10-15 more kernels need to be sectioned, selected and scored.

The score for the sample will be the mean of all sections scored. It should be pointed out that the use of the above factors in estimating standard deviation is to serve only as a guide in determining the number of kernels to be sectioned. For statistical evaluation of results, the conventional method of calculating variance should be used.

Discussion

The individual response of kernels in a grain sample to steeping conditions is quite striking. Some kernels are easily steeped, others are steeped with difficulty; some kernels have much horny endosperm, others have little; in some kernels starch is released from the horny endosperm easily, in others it is held quite tenaciously. The large standard deviations of the samples in Tables I to III are indicative of this individuality. Oxley (2) encountered similar characteristics in his study of the moisture content of individual wheat kernels. Such variability is common among biological materials and here as elsewhere this poses the critical problem of determining a minimum sample size which will provide a score of acceptable reliability.

Many samples of steeped corn and grain sorghum have been examined by the described method. From the results compiled in Tables I to IV the following general observations may be derived:

(1) Samples having low millability scores have high standard deviations. Conversely, the closer a score approaches the limiting value of 100%, the smaller is the standard deviation.

(2) Samples with a large amount of horny endosperm show greater variability than predominantly floury types even at comparable millability scores.

(3) Differences in results obtained by two trained observers are small in comparison with differences among different kernels of a given sample.

Table I shows that steeping time is a factor in variability among

TABLE I

CHANGE IN MILLABILITY SCORE OF A YELLOW DENT CORN WITH STEEPING TIME AS EVALUATED BY TWO OBSERVERS

Hours Steeped	No. Kernels [a] Examined	Horny Endosperm, Percent of Total Area		Observer No. 1		Observer No. 2	
		Observer No. 1	Observer No. 2	Millability Score	Std. Dev.	Millability Score	Std. Dev.
2	[b]						
4	10	16.7	15.7	62.4	±18.5	63.7	±11.3
8	20	13.9		85.8	14.0		
14	10	13.8	9.4	82.5	17.7	85.4	12.8
24	10	10.6	11.6	89.1	6.4	93.4	3.8
32	6	7.3	10.5	90.3	6.3	88.3	13.1
38	6	13.7	10.8	90.1	5.0	90.6	3.9
48	6	15.8	17.7	84.7	14.1	89.3	12.8
56	6	11.8	11.3	86.0	±10.1	88.8	± 7.9
Total Av.	74	13.26	14.61				

[a] The same kernels and sections were scored by both observers.
[b] Too brittle to score.

kernels. The 4 and 8 hour steeped kernels have the lowest scores and the highest standard deviations. Apparently some kernels quickly attain optimum millability while others attain it slowly, thus resulting in a wide range in millability scores for kernels from a sub-optimum-steeped sample. As steeping time increases the slow reacting kernels catch up with the fast reacting kernels, thereby narrowing the gap. The amount of horny endosperm is an important factor contributing to differences in rate of steeping. The horny region is characteristically more slowly steeped than is the floury region but there appear to be other factors. Although broken kernels are rejected in the sampling, it is possible that minute breaks or fissures in the pericarp as well as other physical differences may also be accountable for kernel steeping differences.

It is rather obvious that a variable expressed in percentage units, such as millability score or horny endosperm area, will exhibit decreasing variation as the limiting value of 100 is approached. This, of course, implies that a fixed minimum sample size cannot be applied to all samples. Knowledge of the minimum sample size required for reliable results is important because of the time requirements of the method. Approximately 10 to 20 minutes is required to section, mount, and score each kernel depending upon the skill of the operator and the sharpness of his knife. Therefore, a formula for calculating sample size from coefficient of variation (3) has been applied to these data. At a probability level of 5%, coefficients of variation of 10, 15, and 20% indicated that 19, 44, and 500 kernels would be required, respectively. However, at a probability level of 10% only 3, 7, and 12 kernels would be needed. Obviously, in the interests of practicability, the worker will have to be satisfied with lower precision on samples having coefficients of variation greater than 10%.

To determine more accurately the standard deviation for sub-optimum-steeped samples, a dent corn known to contain a relatively high proportion of horny endosperm was steeped for 8 hours. Fifty kernels were selected at random, sectioned, and scored. The results of a statistical study of the 50 kernels are shown in Table II. In this example, application of the "t" test to mean horny endosperm percentage for randomly selected samples of 10 and 25 kernels verified the null hypothesis. However, with five samples of 10 kernels each, the mean horny endosperm areas and their associated standard deviations were: 23.0 ± 12.2, 25.8 ± 10.6, 24.4 ± 16.8, 23.2 ± 9.4, 24.6 ± 11.9 indicating that a 10 kernel sample in this case would not give an accurate estimate of the standard deviation of the entire sample. Two samples

TABLE II

STATISTICAL DATA ON FIFTY UNSELECTED KERNELS FROM A SAMPLE OF INSUFFICIENTLY STEEPED CORN

	Horny Area, Percent of Endosperm [a]	Horny Starch Removed, Percent of Horny Area	Floury Area, Percent of Endosperm	Floury Starch Removed, Percent of Floury Area	Millability Score
Mean	24.2	30.5	75.8	91.6	70.3
Std. dev., ±	12.0	25.5	12.0	16.5	17.0
Coeff. of var., %	49.5				24.2
Range	0–50	0–90	50–100	50–100	41.4–96.2
Range ÷ 4.5	11.1	20	11.1	11.1	12.2
Conf. limits at 5% level	18.4–30.0				
No. of kernels within conf. limits	27				
Mean	24.8	35.9	75.2	90.3	68.5
Std. dev., ±	4.7	32.0	4.7	10.9	12.5

[a] Columns correspond to symbols H, RH, F, and RF, respectively, given in formula (see section on Procedure).

of 25 each had means and standard deviations of 23.55 ± 11.5 and 24.88±13.0, both of which compared closely with 24.2±12.0 for all 50 kernels.

With such great variability in proportion of horny endosperm it is quite possible to select for scoring a 10 kernel sample skewed on either side of the true value for horny endosperm area. Therefore, close attention must be given to the percentages of horny endosperm in deciding upon the number of kernels which need to be sectioned. For this reason the mean percent horny endosperm area of a sample to be scored should be determined in advance on no less than 25 kernels. From these data, confidence limits can then be calculated which can serve as guides in selecting kernels to score for millability rating which will most truly represent the sample as a whole. This procedure is illustrated in Table II. Out of the 50 kernels examined only 27 fell within the confidence limits of horny endosperm area. The effect of this selection was to reduce the millability score from 70.3 to 68.5 and its standard deviation from 17.0 to 12.5. The reason the standard deviation of the millability score was not reduced more is because of the very great variability in starch removal percentages from horny endosperm. This is a reflection of qualitative differences in response of endosperms in individual kernels to steeping conditions. This particular sample of steeped corn was chosen for study because it was expected to be one of the most variable likely to be encountered.

In Fig. 1 and Table IV are shown exemplary kernels and the scores obtained by two trained observers. These data as well as those in Table I show that differences between two experienced observers are small in relation to the sampling error. Millability of these kernels was also scored by several relatively inexperienced observers who gave values below those shown in Table IV mainly because the amount of horny endosperm was overestimated and the starch removal underestimated. However, the scores of the 6 kernels were in the same relative order.

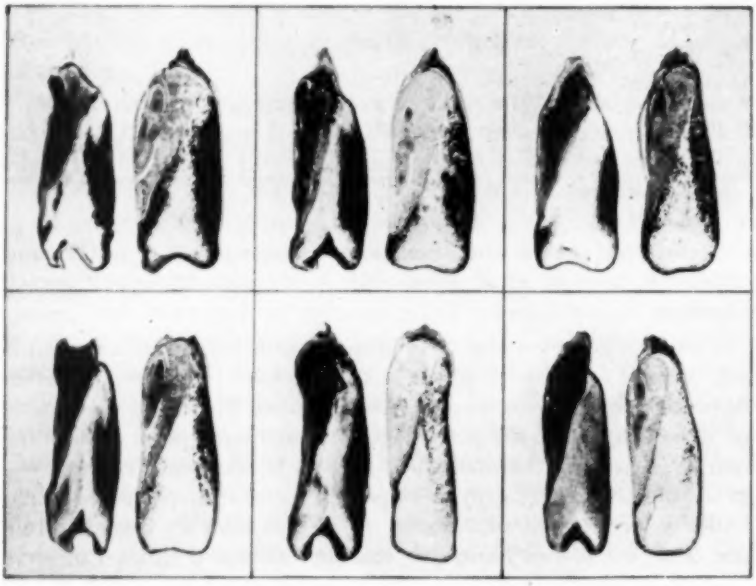

Fig. 1. Longitudinal sections and surfaces of corn kernels representing different degrees of steeping. The left figure in each pair is the brushed, 40μ section stained with I$_2$-KI; right figure is a cut surface to show horny (dark areas) and floury endosperm (lighter areas). Germ areas are on the right side of each kernel. Dark areas at the crown are holes. Scores are given in Table IV.

The estimation of horny endosperm area from the bilateral, longitudinal section probably does not give a correct estimate of relative volumes of horny and floury endosperm because portions located at the sides of the kernel are ignored. However, horny endosperm in dent corn extends as a band around the center of the kernel but in flint corn and grain sorghum the horny endosperm completely surrounds the floury endosperm. Wolf *et al.* (6) state that horny and floury endosperm in dent corn occur in a ratio (percentage by weight) of 2 to

1. Nevertheless, the method of estimating the percentages of horny and floury endosperm areas used in the present paper gives values that are satisfactory for the scoring procedure.

The millability score has been found particularly useful in laboratory comparisons of the steeping and milling properties of different varieties of corn and grain sorghum as shown in Table III. These

TABLE III

COMPARISON OF STEEPING SCORES OF VARIETIES OF CORN AND GRAIN SORGHUM STEEPED 48 HOURS UNDER IDENTICAL CONDITIONS

Type and Variety	No. of Kernels	Horny Area, Percent of Endosperm	Millability Score		
			Mean	Standard Deviation	Coeff. of Variation
Corn					
Yellow Dent	6	11.8	86.0	±10.1	11.7
White Dent, commercial soft	10	4.0	94.2	4.9	5.2
White Dent, commercial medium	10	10.3	91.8	6.5	7.0
White Dent, commercial hard	10	16.2	84.2	19.7	23.4
White Dent, U. of Ill., high-protein	6	33.3	50.1	15.6	31.1
White Dent, U. of Ill., low-protein	6	7.2	96.8	5.9	6.1
Yellow Dent, high-protein hybrid	6	34.2	78.0	15.6	20.0
Yellow Dent, high-oil hybrid	10	29.5	71.5	10.7	15.0
Yellow Flint, Argentine	15	46.7	63.1	21.3	33.8
Grain sorghum					
Martin	6	55.0	79.7	12.3	15.4
Texas Combine Kafir, nonwaxy	6	33.3	92.6	2.8	3.1
Texas Combine Kafir, waxy	6	60.0	83.1	12.3	14.8
D.D. Yellow Sooner, nonwaxy	6	41.7	95.6	1.8	1.9
D.D. White Sooner, waxy	6	36.7	86.1	5.5	6.4
Texas Early Hegari	10	37.0	70.7	11.0	15.6
D.D. Early Shallu	10	32.0	87.7	± 4.9	5.6

TABLE IV

MILLABILITY SCORES OBTAINED BY TWO OBSERVERS FOR KERNELS SHOWN IN FIGURE 1

Kernel No.	Horny Endosperm, Percent of Total Endosperm Area			Starch Removal				Millability Score	
				From Horny		From Floury			
	Observer No.		Pl.[a]	Observer No.		Observer No.		Observer No.	
	1	2		1	2	1	2	1	2
				%	%	%	%		
A	2	2	3	50	50	97	98	95	96
B	20	25	34	80	80	98	98	92	91
C	20	30	39	50	40	95	95	80	70
D	17	18	21	10	10	90	85	67	68
E	35	40	32	20	35	90	90	55	59
F	30	35	38	0	3	85	95	46	47

[a] Area readings with planimeter.

samples, and those in Table I, were steeped under identical conditions using a synthetic steeping medium containing 0.5% lactic acid, 0.15% sulfur dioxide, and sodium hydroxide to give a value of pH 3.0. A water-to-grain ratio of 3.75 ml. per g. of grain (12% moisture) was used which limited the pH rise during the steeping to about 4.2 and provided sufficient sulfur dioxide for complete saturation of the grain. The temperature was 120–125°F. and during steeping the water was circulated over the grain. Grain steeped in this way has been found to give milling results roughly equivalent to commercial steeping.

Differences in millability among individual kernels were observed to be related to the amount of horny endosperm in the kernels. This conforms with previous evidence (1) that the horny endosperm is more slowly steeped and more difficult to mill because the strands of protein binding the starch granules are thicker and less easily broken. There appear to be qualitative differences in the horny endosperm of different varieties and among kernels of a single sample. For example, the Argentine Flint corn shows twice as much horny endosperm as does the Illinois High Protein strain, but the starch is much more readily released from the former as indicated by the higher millability score. The apparent reason is that the cells are smaller and the protein matrix is thicker throughout both the horny and floury portions of the Illinois High Protein kernels than they are in flint or regular dent kernels. This emphasizes the fact that differences between "floury" and "horny" endosperm are differences of degree of starchiness and therefore cannot be precisely defined.

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AN IMPROVED SMALL-SCALE DOUGH EXPANSION TEST FOR THE ESTIMATION OF WHEAT QUALITY[1]

H. Miller, J. Edgar, and A. G. O. Whiteside

ABSTRACT

An improved small-scale dough expansion test involving changes in the apparatus and methods previously described by the authors has been developed. A new all-metal head assembly and jar provide a satisfactory method of sealing the system securely and of holding the dough under test with a minimum of external support. A dough, mixed from 5 g. of flour using a suitable formula, is fermented in air for 45 minutes before being molded into a ball and placed in the expansion apparatus.

In two series of flours involving 187 samples of plant breeders' varieties, correlations ($r = 0.808$ and $r = 0.903$) between loaf volume and expansion were highly significant.

In a previous paper (5) the authors described a dough expansion test using the principle of expanding a fermenting wheat flour dough in a weak salt solution. The expansion of the dough is measured by the displacement of the solution. Several types of equipment have been evolved to measure the expansion of doughs by liquid displacement. The Göttinger apparatus, described by Engelke (2) and modified by Gliemeroth (3), employed much the same principle as did Jongh's (4) more recent apparatus. The Göttinger apparatus allows the displaced liquid to overflow into a graduated cylinder. The dough is held beneath the surface of the liquid by a screen which probably lends some support to the dough mass. Other types of apparatus, using a closed or trap system which measures all the gas, have been described. The present method is in no way concerned with the total amount of gas produced by the dough, either during fermentation in air or while under test.

Description of Apparatus

The original apparatus was an experimental one and it seemed desirable to replace it by a device which would be more durable, more positive, and more convenient in handling. The new apparatus, shown in Fig. 1, is made in three pieces: an aluminum jar (inside dimensions 7 cm. $\times$ 6 cm.), a screw-cap collar of the same material, and an assembly head which is held in position by the collar. The assembly head is made of brass and is nickel-plated. It is slotted to hold a

[1] Manuscript received March 23, 1954. Presented at the Annual Meeting, May, 1954. Paper No. 179 of the Cereal Crops Division, Experimental Farm Service, Canada Department of Agriculture, Ottawa.

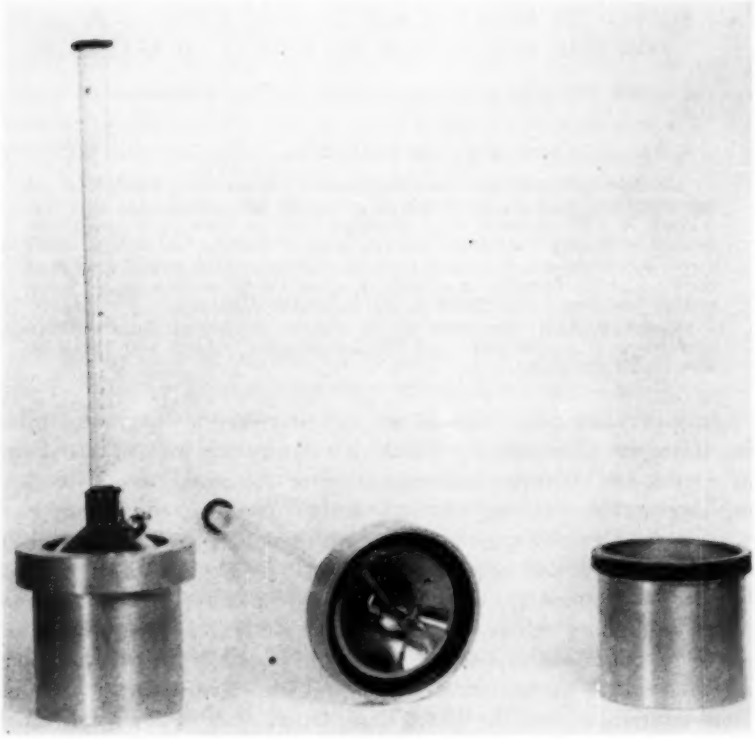

Fig. 1. Apparatus for measuring expansion.

standard gem-jar rubber ring which makes the seal with the rim of the jar. The cruciform dough holder, so constructed as to give minimum support to the fermenting dough, is attached to the underside of the head. The precision bore tube is sealed into the neck of the assembly head. A small release valve to adjust the brine solution to zero at the beginning of each test is inserted into the side of the assembly head. A mixer capable of handling 5-g. flour doughs was used but since this is undergoing further modifications it will be described in a future publication.

Methods

Two liquids are made up for the test and the quantities shown are sufficient for 40 tests. The phosphate-bromate stock solution is made by dissolving 50 g. of monoammonium phosphate and 0.5 g. of potas-

sium bromate in 500 ml. of distilled water.

Salt-sugar solution		*Yeast suspension*	
Sodium chloride	1.0 g.	Yeast–baker's comp.	12.0 g.
Dextrose	3.0 g.	Water, distilled	40.0 ml.
Sucrose	5.0 g.		
Phosphate bromate (Stock)	1.0 ml.		
Water, distilled	59.0 ml.		

Five-gram samples of flour are weighed into 1-oz. wide-mouth bottles fitted with screw caps. To each 5.0-g. sample placed in the mixing bowl are added 1.61 ml. of salt-sugar solution, 1.10 ml. of yeast suspension, and enough distilled water, usually from 0.2 to 0.4 ml., to produce a dough of satisfactory consistency. Pre-set automatic pipettes are used to dispense the required amount of liquids. Strong flours are mixed for 1 minute and weak flours for 45 seconds or less. The dough is rounded by hand and placed on a sheet-metal holder in a covered jar partially filled with 0.5% solution of sodium chloride to maintain a high humidity. These jars are held in a water bath controlled at 37°C. After 45 minutes' fermentation the dough is molded into a ball and impinged on the dough holder of the assembly head. The expansion jar is filled with 0.5% sodium chloride solution; the head and collar are screwed on tight. After the sodium chloride solution has been leveled to zero, the expansion unit is placed in the water bath held at 37°C. This whole operation should be completed rapidly, as the doughs at this stage are undergoing active fermentation. The measure of expansion is recorded as the maximum height, in ml., to which the brine rises in the measuring tube.

In practice, one man working alone can make thirty tests a day, a number which could be greatly increased with the help of an assistant.

Experimental Application of the Expansion Test

The replicability of the test was examined in a study of 72 tests on each of two different flours. The standard error of a single determination for flour A was 0.393 and for flour B, 0.435, and the corresponding means were 16.94 and 16.51. From these data it is suggested that the limits for checking duplicates would be about 1.1 ml. since a significant difference between two single determinations would be approximately $2 \times \sqrt{2} \times 0.4 = 1.1$, where 2 represents a t value at the 5% point, $\sqrt{2}$ converts the standard error of a single determination to the standard error of a difference, and 0.4 is a close approximation of the standard error of a single determination.

The usefulness of the expansion test is measured by its ability to segregate wheats into high, medium, and low quality in breeding programs where parents differ widely in baking quality as measured by loaf volume. Two series of wheats of this type from the Cereal Breeding Laboratory at Winnipeg were studied. The first series, the preliminary rod row test, contained 107 samples, and the second, the general rod row test, contained 80 samples. Both series were composites of material from five stations. Wheat protein and loaf-volume data, the latter by the malt-bromate-phosphate method (1), were aach correlated with the expansion figures. For comparison, correlation coefficients were caluculated for wheat protein and loaf volume. High, low, and mean values of each series are given in Table I.

TABLE I

MAXIMA, MINIMA, AND MEANS FOR WHEAT PROTEIN, LOAF VOLUME, AND EXPANSION

Series and No. of Samples	Loaf Volume	Expansion	Wheat Protein
	cc.	*ml.*	%
1—107 samples			
Maximum	815	18.3	16.1
Minimum	465	11.1	13.1
Mean	656	14.7	14.6
2—80 samples			
Maximum	860	20.4	15.6
Minimum	445	8.6	12.3
Mean	695	15.8	14.2

TABLE II

CORRELATION COEFFICIENTS FOR THE TWO SERIES OF FLOURS

	Series 1	Series 2
Loaf volume and expansion, r_{12}	0.808**	0.903**
Loaf volume and wheat protein, r_{13}	—0.097	0.341**
Expansion and wheat protein, r_{23}	0.036	0.432**

**Significant at 1% level.

In Table II the correlation coefficients for the two series of flours are given. The highly significant correlations for loaf volume and expansion indicate that expansion was a good index of loaf volume and much better than wheat protein. This is illustrated in Figs. 2 and 3 where data for Series 2 are shown graphically. For Series 1 little relationship between loaf volume and wheat protein was obtained. The correlations for expansion and wheat protein were similar to those for loaf volume and wheat protein for both series.

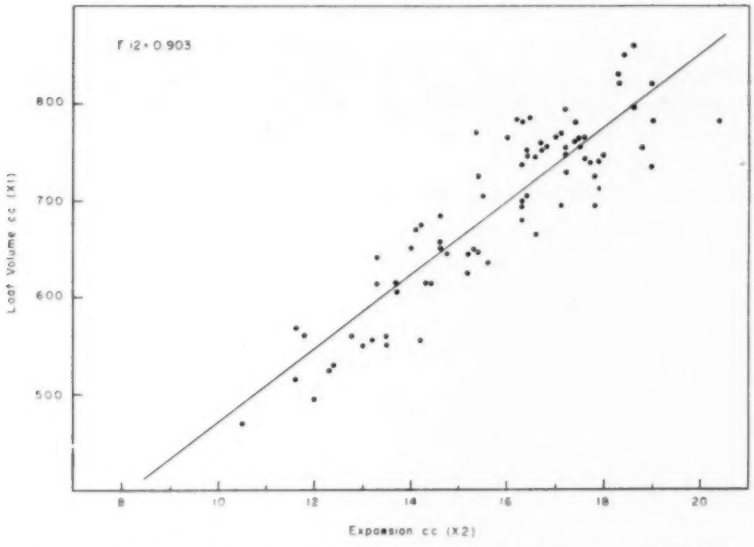

Fig. 2. Relationship of loaf volume to expansion in general rod row series, Winnipeg.

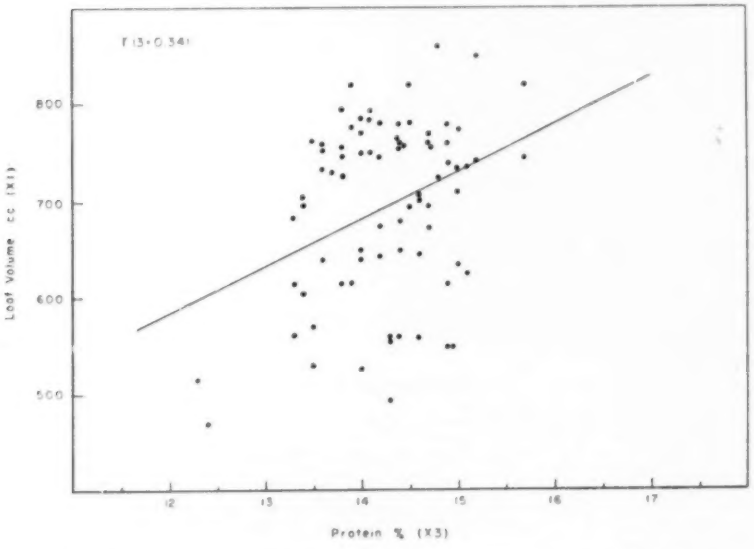

Fig. 3. Relationship of loaf volume to wheat protein in general rod row series, Winnipeg.

Discussion

The expansion test appears to offer a simple method of testing small samples of wheat for potential loaf volume. So far, only flour doughs have been tested since the work has been confined largely to determining the relation between the expansion figure of a flour dough and the volume of a loaf baked from the same flour. The method is simple and suitable for routine testing of large numbers of small samples. By the use of a system where preliminary fermentation precedes the expansion test, a closer analogy to the baking test is obtained than may otherwise be possible without this pre-fermentation.

Acknowledgments

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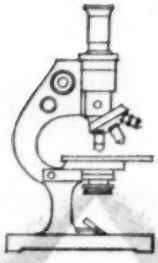

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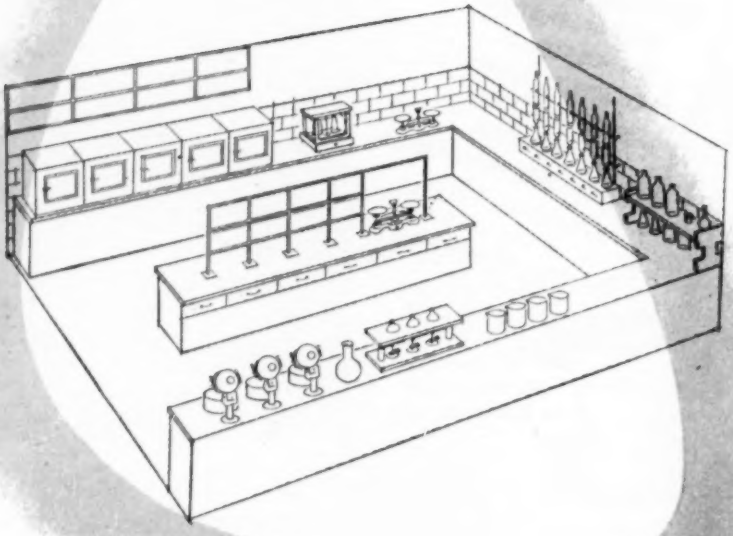

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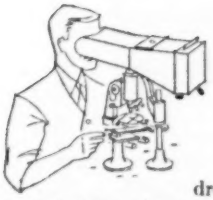

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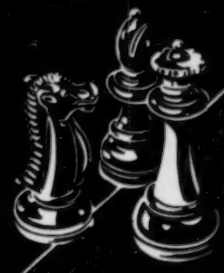

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